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(54) Title: NUCLEIC ACID VACCINES USING TUMOR ANTIGEN ENCODING NUCLEIC ACIDS WITH CYTOKINE ADJUVANT ENCODING NUCLEIC ACID

(57) Abstract: Nucleic acid vaccines are provided that comprise at least one tumor antigen encoding nucleic acid and at least one cytokine adjuvant encoding nucleic acid for prophylaxis or treatment of tumors. The viral vaccines of the invention are optionally combined or additionally administered with a recombinant virus or DNA vaccine booster.

NUCLEIC ACID VACCINES USING TUMOR ANTIGEN ENCODING NUCLEIC ACIDS WITH CYTOKINE ADJUVANT ENCODING NUCLEIC ACID

FIELD OF THE INVENTION

The present invention relates to nucleic acid vaccines comprising sequences that encode a tumor antigen as an immunogen and a cytokine as an adjuvant. The vaccines are suitable for the vaccination of mammals, including humans, in order to provide unexpectedly enhanced cellular and/or humoral immune responses to one or more tumor related pathologies. Additionally, the invention relates to methods for making and using such nucleic acid vaccines.

BACKGROUND OF THE INVENTION

Cancer is a serious disease that afflicts one in four people. In the last fifty years, there have been significant improvements in the early detection of cancer, as well as the development of a number of therapies to treat cancer. Therapies include surgery to remove primary tumors, and sublethal radiation and chemotherapy to treat disseminated disease. While these treatments have resulted in apparent cures for many patients, the treatments can be quite debilitating and are still often ineffective at preventing death from this disease. There is clearly a need for therapies that are less destructive, as well as for novel therapies that harness the body's natural defenses to fight cancer.

Cancer can be divided into two classifications, depending upon the cell type the tumor is derived from. For example, carcinomas are derived from epithelial cells, while sarcomas are derived from mesodermal tissues. Some epithelial tumors express on their surface a protein called mucin 1 (MUC1).

MUC1 is a transmembrane protein that is normally expressed in non-disease states on ductal epithelial cells, such as those in the intestinal mucosa exposed to the lumen of the small intestine. The most notable feature of MUC1 is its large extracellular domain, which is comprised of 30-100 tandem repeats of a 20 amino acid sequence. The tandem repeats confer a rigid structure to this portion of the protein, and the repeats are a substrate for heavy glycosylation. In addition, in normal cells MUC1 is only expressed on the ductal side of the cell. It is thought that MUC1 may provide a lubrication function to the duct, and it may also be involved in signal transduction. Because the protein is normally expressed on the ductal side of cells, it is rarely exposed to the outside of the organism, and is considered a "sequestered antigen", because in its native form MUC1 is not exposed to immune system surveillance.

In contrast, MUC1 expression is different in epithelial tumors. The protein becomes overexpressed and is present all over the surface of the cell, and it is relatively deglycosylated as compared to the normal form expressed in ductal epithelial cells. Thus, the distribution and pattern of expression is very different in normal and neoplastic tissues, and the deglycosylated, aberrant protein exposes novel epitopes to the immune system. Because the pattern of expression is different from normal, it is possible that the immune system can now recognize the tumor-associated MUC1 as foreign and attempt to destroy the cells expressing this protein. Indeed, the immune system does appear to act in this way in some cancer patients. It has been shown that patients with ovarian, breast or pancreatic cancer possess weak antibody and cytotoxic T lymphocyte (CTL) responses to MUC1, indicating that their immune systems do indeed recognize a difference in the tumor-associated MUC1. However, the immune responses are clearly not strong enough to eliminate tumor cells.

These observations have led some investigators to develop therapeutic strategies designed to induce or strengthen the natural immune response. For example, several groups have attempted to use MUC1 peptides to prime a cellular response in patients. This relies on the concept that cells could process the peptide and present it in the context of Class I molecules to the immune system, to cause a Th1 response to cells expressing the MUC1 protein. There are several disadvantages to known approaches. First, peptides have short half-lives, requiring administration of large amounts of the peptide. Second, each person expresses several Class I molecules and a given peptide binds to only one molecule, which will be held by a minority of the patient population. Third, the immunity generated by such approaches may not be relevant to treating such cancers; it has been noted that anti-peptide immunity can be generated by peptide immunization, which does not always lead to anti-protein immunity.

The identification of tumor-specific antigens has supported the concept that immunologic strategies could be designed to specifically target tumor cells in cancer patients. Immunologic recognition of tumor antigens has been subsequently documented in patients with malignancy. However, these responses are muted and are ineffective in eradicating disease. The development of immune tolerance towards malignant cells is due, in part, to the inability of tumor cells to effectively present antigens to the immune system. Therefore, T cells with the capability of recognizing these antigens fail to become activated. A major focus of cancer immunotherapy has been the attempt to introduce tumor antigens into the cancer bearing host such that they may be recognized more effectively and that meaningful antitumor responses can be generated. In this way, native immunity directed against antigens selective for or over-expressed in malignant cells may be amplified and result in tumor rejection. Approaches to induce tumor-specific immunity have included vaccination with tumor cell extracts, irradiated cells, tumor-specific peptides with

and without adjuvant, and dendritic cells (DC) pulsed with tumor peptides/proteins, or manipulated to express tumor-specific genes.

DNA immunization has been used as a method to generate immune responses *in vivo*, and has been recognized as an effective way to generate cytotoxic T cells directed against an encoded antigen. Vaccination with tumor-specific naked DNA results in the expression of tumor antigens by the inoculated muscle cells. Professional antigen presenting cells, in particular DC, recruited to the site of injection, internalize and subsequently present the tumor-specific antigens at sites of T-cell traffic.

Breast cancer is a common malignancy second only to lung cancer among cancer deaths in women. In 2000, it was estimated that 182,800 new cases were diagnosed and 41,200 deaths resulted from breast cancer in the United States (US). Standard-dose combination chemotherapy can yield high response rates in previously untreated patients with metastatic disease, but complete responses are rare. Despite initial chemosensitivity, median disease response duration is less than 1 year due to the emergence of chemoresistant disease. The median survival for patients with metastatic disease has remained approximately 2 years for those treated with standard-dose chemotherapy. A majority of breast carcinomas express MUC1. As noted in the Investigator's Brochure, responses to recombinant vaccine constructs expressing MUC1 have been shown to induce immune responses in mice and chimpanzees. As such, immunotherapeutic strategies targeting the MUC1 antigen are a potentially promising approach for patients with metastatic breast cancer who otherwise lack effective treatment options.

Prostate cancer is the second leading cause of cancer-related death in men. Approximately 180,000 men will be diagnosed with prostate cancer each year, and 40,000 succumb to the disease each year. Prostate tumor cells have a low proliferation rate and do not respond to standard chemotherapies, which are most toxic to the most rapidly dividing cells in the body. Instead, prostate cancer can be treated surgically, with radiation therapy or hormonal therapy. Surgery and radiation therapy can lead to undesirable side effects, such as incontinence and impotence. The disease can often be successfully managed with hormonal therapy, which starves the cells for its required growth factors. However, eventually all tumors treated in this way become androgen-independent and there is no effective treatment beyond that point. There is clearly an unmet medical need to treat this disease more effectively, and with novel therapies.

One such approach that has considerable promise is active immunotherapy. Active immunotherapy would stimulate the patient's immune system to generate an anti-tumor response that could help hold the disease in check longer, or even rid the patient of metastatic disease. One

example of active immunotherapy include dendritic cell therapies, where the patient's professional antigen presenting cells are removed and pulsed with tumor antigen, transfected with tumor RNA/cDNA, or fused with tumor cells. The ex vivo-treated dendritic cells are then reinjected into the patient, and are expected to drive a prostate-tumor specific immune response. One disadvantage of such approaches is that they amount to designer therapy that would be very costly and require very specialized skills to administer. Such therapies are unlikely in their current form to be widely used.

A second active immunotherapy approach is peptide vaccination. In this approach, tumor-specific peptides or proteins are administered to the patient, with the hope of directly loading antigen-presenting cells in vivo. This approach is more likely to be usable in the clinic than the ex vivo approach described above, but consistent success has not yet been achieved with this strategy. Some problems include that fact that peptides are short-lived in vivo, and therefore require very large doses. In some clinical trials, peptide vaccination engenders anti-peptide immune responses that do not translate into responses against tumors expressing the whole protein from which the peptides were derived.

A third active immunotherapy approach that has much more promise to be widely used would be a cancer vaccine. Specifically, we believe that a DNA vaccination approach could be very effective in treating prostate cancer patients. In this treatment, the vaccine would be comprised of plasmids (or other DNA-containing agents) that encode antigen(s) specific to prostate cancer. The plasmids would be injected into the patient, and the prostate-specific antigens would then be expressed and presented to the immune system. The antigen-presentation process would engender a specific cellular and/or humoral response that could help to control the growth of the tumor or its metastases. From preclinical models there is reason to believe that such an approach could be effective. For example, vaccination of rhesus monkeys with DNA vaccines encoding PSA +/- cytokine adjuvants drives PSA-specific humoral responses and cellular proliferation. In two male monkeys vaccinated in this way, there was evidence of infiltrating cells within the prostate post vaccination, but not in a nonvaccinated control. In work in our labs, we have shown that vaccination with DNA encoding a different tumor associated antigen, MUC1, can lead to immune responses protective against tumor challenge with MUC1-expressing tumors. Thus, it may be possible to use DNA vaccines to break tolerance to self-antigens that happen to be strongly expressed by tumors, and mount a therapeutic immune response.

While vaccination with PSA with or without cytokine adjuvants may very well be effective as an immunotherapy, it is possible that this would not be enough.to control tumor growth. It is entirely

possible that an effective immune response against PSA would eliminate PSA+ tumor cells but leave PSA- prostate tumor cells intact and able to grow unfettered. Therefore, it may be desirable to vaccinate with more than one tumor antigen. We propose that a DNA vaccine comprised of the PSA antigen with other antigens expressed highly in prostate cancer, such as KLK2 and/or MUC1, and perhaps with other adjuvant/costimulatory genes, would be a more effective approach than vaccination with a single antigen.

PSA or KLK3 is a member of a multigene family known as the human kallikrein gene family. There are 15 closely related genes in the family, all of which map to a 300kb region of human chromosome 19q13.3-q13.4. Kallikreins are secreted serine proteases. All are synthesized as preproenzymes; proenzymes arise after removal of the signal peptide, and the mature active protease arises after removal of a propeptide. The activity of a given kallikrein will be either trypsin-like or chymotrypsin-like, depending upon the nature of the active site. PSA or KLK3 is a 30 Kd serine protease with chymotrypsin-like activity, which is responsible for cleaving seminogelin I, seminogelin II and fibronectin in seminal fluid. PSA is most highly expressed in the prostate, but it is also expressed at lower levels in breast, salivary gland, and thyroid. Besides prostate cancer, PSA is expressed in some breast malignancies. PSA has become well known as a serum marker for prostate cancer; it is a very important diagnostic for this disease and increasing serum levels of PSA typically correlate well with the severity of the disease. Expression of PSA is not increased in prostate cancer cells versus normal prostate cells; instead as the disease breaches the normal cellular barriers, PSA leaks into the serum. It is unclear if PSA has a role in the etiology of prostate cancer; various reports have indicated that PSA could either enhance or inhibit tumorigenicity. Several CTL epitopes for PSA have been described for the HLA A2 and A3 haplotypes; identification of these epitopes support the possibility of generating therapeutic in vivo CTL by vaccination.

KLK2 is the member of the kallikrein family that most closely resembles PSA, with about 80% identity at the amino acid level. Like PSA, KLK2 is expressed highly in the prostate and in prostate cancer, with lower levels of expression in other tissues, such as breast, thyroid, and salivary gland. KLK2 has trypsin-like activity, and one of its activities is to cleave the proenzyme form of PSA to yield the mature enzyme. There is increasing recognition that KLK2 may be a good serum prognostic indicator to monitor the progress of prostate cancer patients, although it is likely to be a supportive diagnostic along with PSA.

Accordingly, there is a long-felt and pressing need to discover vaccines and methods that elicit an immune response that is sufficient to treat or prevent various tumor related human pathologies.

SUMMARY OF THE INVENTION

The present invention is intended to overcome one or more deficiencies of the related arts. In particular, nucleic acid vaccines of the present invention advantageously provide a more robust immune response. The strength of the present invention lies in its power to recruit one or more of B cell, helper T cell, and cytotoxic T cell components of the immune response for effective humoral and cellular immunity.

To provide more effective tumor or cancer vaccines, the present invention provides nucleic acid vaccines comprising a cancer-specific or tumor-specific antigen nucleic acid and an adjuvant nucleic acid. Also provided are methods of making and using such nucleic acid vaccines. In their use as a vaccine, the co-expression of tumor nucleic acid and the adjuvant nucleic acid in a tissue to which the vaccine of the present invention has been introduced induces a cellular or humoral immune response, or any component thereof, to the tumor protein or fragment thereof.

This invention uses nucleic acids (or fragments thereof) encoding such tumor antigens as, but not limited to, prostrate specific antigen (PSA), KLK2, and/or mucin-1 (MUC1) as antigen components of a DNA vaccine for tumors, such as but not limited to, any PSA, KLK2 or MUC-1 associated tumor or cancer. The antigen genes will be of human origin, or mutated to enhance their immunogenicity. Examples of how the antigen genes could be rendered more immunogenic would include alteration or removal of signal sequences required for secretion, optimization of codons for improved translation, addition of ubiquitination signals for degradation, addition of subcellular compartment targeting sequences, addition of molecular chaperone sequences, and optimization of CTL epitopes. The antigen genes could be fused together to increase immunogenicity. The CTL/helper epitopes could be linked together, or inserted as part of another molecule, such as an immunoglobulin molecule.

Other genes may also be included in the vaccine, including cytokine adjuvant genes such as IL-18, IL-12 or GM-CSF, or genes for costimulatory molecules such as B7-1, which would help to drive the immune response.

The genes of the invention could be encoded by plasmids, viruses, bacteria or mammalian cells. The vaccination regimen could be comprised of any or all of these agents, such as a plasmid DNA

priming vaccination, followed by a viral vector boost. The latter approach appears to be effective in generating cellular responses important in controlling infectious diseases (28-32), and may be very useful in anti-cancer applications of this technology as well.

In the vaccines of the invention, the tumor encoding nucleic acid may be isolated from patients having a tumor related cancer, preferably from the cancerous tissue itself or from mRNA or cDNA encoding a cancer-related tumor protein or antigenic portion thereof.

The present inventors have discovered that nucleic acid vaccines of the present invention elicit unexpectedly enhanced immune responses by the expression and/or presentation of at least one tumor antigen encoding nucleic acid and at least one cytokine adjuvant encoding nucleic acid.

The present invention also provides at least one tumor/adjuvant nucleic acid encoding (or complementary to) at least one antigenic determinant encoding nucleic acid of at least one tumor protein and at least one adjuvant encoding nucleic acid of at least one portion of an IL-18 protein.

The present invention also provides a tumor/adjuvant vaccine composition comprising a tumor/adjuvant nucleic acid vaccine of the present invention, and a pharmaceutically acceptable carrier or diluent. The vaccine composition can further comprise an additional adjuvant and/or cytokine encoding sequence or further component of the composition which enhances a nucleic acid vaccine immune response to at least one cancer associated tumor protein in a mammal administered the vaccine composition. A nucleic acid vaccine of the present invention is capable of inducing an immune response inclusive of at least one of a humoral immune response (e.g., antibodies) and a cellular immune response (e.g., activation of B cells, helper T cells, and cytotoxic T cells (CTLs)), with a cellular immune response preferred.

The present invention also provides a method for eliciting an immune response to a cancer associated tumor protein in a mammal which is prophylactic for a cancer associated tumor protein, the method comprising administering to a mammal a vaccine composition comprising a nucleic acid vaccine of the present invention, which is protective for the mammal against a clinical MCU-1-related pathology.

The present invention also provides a method for eliciting an immune response to a cancer associated tumor protein in a mammal for therapy of a tumor-associated pathology, such as but

not limited to a tumor or cancer. The method comprises administering to a mammal a composition comprising a nucleic acid vaccine of the present invention, which composition elicits an enhanced immune response, relative to controls, in the mammal against a clinical tumor related pathology.

In a further embodiment, the prophylactic or therapeutic method of eliciting an immune response to tumor comprising administering an effective amount of another (e.g., second) nucleic acid vaccine comprising at least 1 to about 100 different tumor protien fragments or variants, in which the fragments or variants relate to different tumor nucleic acid or amino sequences, preferably related to a cancer-associated or pathology-associated tumor protien or antigen sequence.

The tumor-specific immune response generated with at least one nucleic acid vaccine of the invention can be further augmented by priming or boosting a humoral or cellular immune response, or both, by administering an effective amount of at least one tumor/adjuvant vaccine. Any of the vaccine strategies provided herein or known in the art can be provided in any order. For example, a subject may be primed with a nucleic acid vaccine, followed by boosting with a nucleic acid vaccine or a protein vaccine. Preferably, the tumor/adjuvant vaccine is administered intramuscularly. Preferably, the vaccine is in the form of a plasmid and is administered with a gene gun or injector pen, needled or needleless. However, other forms and administration are also suitable and included in the present invention.

The present invention also provides methods, compositions, articles of manufacture and the like, for making and using a tumor/adjuvant nucleic acid vaccine of the present invention.

Other objects, features, advantages, utilities and embodiments of the present invention will be apparent to skilled practitioners from the following detailed description and examples relating to the present invention, in combination with what is known in the art.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Female C57Bl/6 mice were vaccinated three times (Day –28, -14, and –7) with buffer, empty vector, pMUC1 plasmid, pIL-18 plasmid, or combinations of the latter two plasmids. Animals were challenged with MUC1+ mouse tumor cells on Day 0, and were monitored for tumor incidence for 50 days.

Figure 2. Female C57Bl/6 mice were vaccinated three times (Day –28, -14 and –7) with buffer, empty vector, pMUC1 plasmid, pIL-18 plasmid, or combinations of the latter two plasmids. Animals were challenged with MUC1+ mouse tumor cells on Day 0, and were monitored for tumor growth for up to 50 days.

Figure 3. C57Bl/6 mice free of tumors in Figure 1 were rechallenged with $MUC1^+$ tumor cells on Day 49 (denoted Day 0 in this figure). Mice were monitored an additional 49 days after the second tumor challenge.

Figure 4. MUC1 Tg mice were vaccinated three times (Day –28, -14, and –7) with the plasmids indicated in the legend. Mice were challenged with MUC1+ tumor cells on Day 0 and monitored for tumor incidence for 28 days.

Figure 5. Animals from Figure 4 were sacrificed, and their tumors were excised and weighed on Day 28 after tumor challenge. Horizontal bars are median values.

Figure 6. Phase II of the pMUC1/pIL-18 vaccination of MUC1 Tg mice. MUC1 Tg mice without tumors at the end of Phase I (Figure 4) were rechallenged with a second dose of MUC1+ tumor cells on Day 50 after the first challenge (denoted Day 0 in this figure). Mice were monitored for tumor incidence for 28 days after the second challenge.

Figure 7. Remaining tumor-free MUC1 Tg mice from Phase II (Figure 6) were challenged on Day 28 of Phase II with $MUC1^-$ parental tumor cells (denoted as Day 0 in this figure). Animals were monitored for tumor incidence 39 days post challenge.

Figure 8A-C. A. DNA sequence of human IL-18plasmid p1968 with the protein sequence of Figure 8B included. **B, C.** Protein sequence of the precursor human IL-18 produced by the engineered IL-18 constructs. The first 19 residues are derived from the 12B75 HC signal sequence; the remaining 161 residues are the mature human IL-18. In the version shown in C, the first residue of the mature human IL-18 sequence is altered to better conform to consensus human immunoglobulin signal sequences.

Figure 9A-D: Sequence of human MUC1 cDNA with intron 6 incorporated.

Figure 10. Tumor incidence in female MUC1 transgenic mice vaccinated with DNA as indicated in the legend, and subsequently challenged with $MUC1^+$ tumor cells. Only the group vaccinated with pMUC1/pIL-18 shows significantly improved protection from tumor challenge ($p=0.007$).

Figure 11. Media tumor weights at study end, from animals shown in Figure 1. Media tumor weight for group 4 is significantly different from those in the other groups.

Figure 12. Rechallenge of protected mice from Figure 1 with MUC1⁻ tumor cells.

Figure 13. Tumor incidence in male mice vaccinated with pMUC1 or empty vector, followed by tumor challenge.

Figure 14. Tumor weights in male mice vaccinated with pMUC1.

Figure 15. Tumor incidence in male mice rechallenged on the opposite flank with MUC1+ tumor cells.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present inventors have discovered that unexpectedly enhanced immune responses can be induced against tumor associated pathologies, by the use of nucleic acid vaccines that contain a combination of at least one tumor antigen or protein encoding nucleic acid and at least one cytokine encoding nucleic acid.

The terms "priming" or "primary" and "boost" or "boosting" are used herein to refer to the initial and subsequent immunizations, respectively, i.e., in accordance with the definitions these terms normally have in immunology.

The component encoding nucleic acids of a tumor/adjuvant encoding nucleic acid of the present invention can be provided using any known method or source. Alternatively, the different tumor nucleic acids can be obtained from any source and selected based on screening of the sequences for differences in coding sequence or by evaluating differences in elicited humoral and/or cellular immune responses to multiple tumor sequences, in vitro or in vivo, according to known methods.

As is readily appreciated by one of skill in the art, the inventors have further found that boosting with a tumor/adjuvant vaccine of the present invention further potentiates the immunization methods of the invention. The tumor protein(s) encoded by the nucleic acid vaccine can be similar or different different to the tumor protein(s) in the boosters.

Similarly, as can be appreciated by the skilled artisan, the immunization methods of the present invention are enhanced by use of primer, booster or additional administrations of a DNA vaccine of the present invention. The tumor/adjuvant vaccine can be used as a boost, e.g., as described above with respect to the tumor proteins. Alternatively, the vaccine can be used to prime immunity, with the vaccine or vaccines used to boost the anti-tumor immune response. The vaccine may comprise one or more vectors for expression of one or more tumor proteins or portions thereof. In a preferred embodiment, vectors are prepared for expression as part of a

DNA vaccine.

The invention is a therapeutic vaccine that would be used in patients with cancer, where PSA and/or KLK2 and/or MUC1 are uniquely expressed, or overexpressed relative to normal tissue. The vaccine could potentially be preventative therapy for individuals at high risk of developing prostate or other cancers or tumors expressing these antigens. The vaccine could also be used in other cancers where PSA and/or KLK2 and/or MUC1 are either uniquely expressed or overexpressed relative to normal tissue. The vaccine would be comprised of DNA encoding any combination of these antigens, and could be contained within one or more plasmids, mammalian viruses, bacteria or mammalian cells. The antigen or adjuvant encoding nucleic acids as one or more components of the vaccine could include any alternatively spliced forms that naturally occur. The antigen genes may contain modified sequences that will include optimized codons for translation in human cells, or signals for ubiquitination that would lead to enhanced degradation. The vaccine could contain fragments of the antigen genes, including antigen-specific CTL epitopes linked to each other, or to other heterologous CTL epitopes and/or homologous/heterologous CD4 helper epitopes. Fragments of the antigen genes could be generated that lack signal sequences, which could enhance degradation and antigen presentation. Fragments of the antigen genes could be encoded as fusions with other proteins, or inserted within other protein sequences, such as immunoglobulin sequences. Natural variant sequences have been reported for PSA, KLK2 and MUC1, and are useful in the present invention, e.g., but not limited to those presented in SEQ ID NOS:1-47, and specified variants thereof.

The vaccination regimen could include a mixture of DNA-encoding agents, temporally administered in different orders, or administered in different places in the body at the same time. Plasmids could be formulated in lipid, buffer or other excipients or chemical adjuvants that could aid delivery of DNA, maintain its integrity in vivo, or enhance the immunogenicity of the vaccine. The vaccine could also be delivered by direct injection into muscle, skin, lymph node, or by application to mucosal surfaces. Other potential modes of delivery would include injection of DNA, followed by electroporation to enhance cellular uptake and expression of DNA.

One possible cytokine adjuvant that could be included in the vaccine is human IL-18. Variants of human IL-18 sequence have been reported, , e.g., but not limited to those presented in SEQ ID NOS:60-77, and specified variants thereof. The macaque sequence for IL-18 is very similar to human IL-18, and can also be used according to the present invention.

The antigen genes, or costimulatory molecule genes, or cytokine adjuvant genes would be expressible in humans because of being linked to a promoter. The genes would also be expressible because of linkage to a polyadenylation signal, such as the SV40 late polyadenylation signal. An intron may be included for enhanced expression, such as the HCMV IE intronA, or natural introns from the antigen or adjuvant genes.

Advantages:

Active immunotherapy offers the possibility that cancer patients could develop long-lasting and vigorous immune responses against their tumors that would prolong life, slow disease progression, and possibly eradicate disease. When used as an adjunct therapy, active immunotherapy may increase quality of life by minimizing the toxicity of other conventional therapies. DNA vaccination in particular offers a simple approach toward generating protective immune responses.

We have demonstrated in our MUC1 vaccination model that DNA vaccination can lead to epitope spreading. There are no other reports of anti-tumor efficacy engendered by coadministration of plasmid DNA encoding MUC1 and any other costimulatory/adjuvant molecule, particularly IL-18. In addition, this is the only instance found so far of epitope spreading as a result of plasmid DNA vaccination in tumor models. As mentioned above, if this phenomenon could be induced in humans, it would induce immunity to MUC1 as well as to other unknown tumor-associated antigens that are present in the tumor. This multi-antigen attack on the tumor would minimize or inhibit the ability of the tumor to evade the immune response. This approach also is applicable to a vaccine using PSA as the antigen, or PSA in combination with other antigens and adjuvant molecules.

Another advantage of our approach is the ability to encode more than one gene on a plasmid or DNA vehicle to enable delivery of more than one protein product to a target tissue/cell (33, 34). This should ensure that a target tissue expresses all desired proteins with the expectation of a more efficient induction of immune response. For example, we have constructed a double cistron vector, and for example we have shown that it is capable of expressing mouse or human IL-12. IL-12 is a protein comprised of two subunits that must be co-expressed in the same cell in order for the mature molecule to be produced. The two protein subunits are encoded by different genes, and we have shown in tissue culture that a double cistron vector encoding both genes results in more effective production of the mature protein than using two plasmids which encode either gene alone (33, 34).

Nucleic acid vaccines and Vaccination

The present invention thus provides, in one aspect, nucleic acid vaccines using mixtures of at least 1, and up to 50 different tumor and cytokine encoding nucleic acids that optionally each can express a different protein variant, or an antigenic portion thereof. As can be readily appreciated to one of skill in the art, 1 to about 50 different tumor protein encoding nucleic acids can be employed. Also provided are methods of making and using such nucleic acid vaccines.

A nucleic acid vaccine of the present invention induces at least one of a humoral and a cellular immune response in a mammal who has been administered at least one nucleic acid vaccine, but the response to the vaccine is subclinical, or is effective in enhancing at least one immune response to at least one tumor antigen, such that the vaccine administration is suitable for vaccination purposes.

DNA vaccines. An alternative to a traditional vaccine comprising an antigen and an adjuvant involves the direct in vivo introduction of DNA encoding the antigen into tissues of a subject for expression of the antigen by the cells of the subject's tissue. Such vaccines are termed herein "DNA vaccines" or "nucleic acid-based vaccines." DNA vaccines are described in International Patent Publication WO 95/20660 and International Patent Publication WO 93/19183, the disclosures of which are hereby incorporated by reference in their entireties. The ability of directly injected DNA that encodes a viral protein to elicit a protective immune response has been demonstrated in numerous experimental systems (Conry et al., Cancer Res., 54:1164-1168 (1994); Cox et al., Virol, 67:5664-5667 (1993); Davis et al., Hum. Mole. Genet., 2:1847-1851 (1993); Sedegah et al., Proc. Natl. Acad. Sci., 91:9866-9870 (1994); Montgomery et al., DNA Cell Bio., 12:777-783 (1993); Ulmer et al., Science, 259:1745-1749 (1993); Wang et al., Proc. Natl. Acad. Sci., 90:4156-4160 (1993); Xiang et al., Virology, 199:132-140 (1994)). Studies to assess this strategy in neutralization of influenza virus have used both envelope and internal viral proteins to induce the production of antibodies, but in particular have focused on the viral hemagglutinin protein (HA) (Fynan et al., DNA Cell. Biol., 12:785-789 (1993A); Fynan et al., Proc. Natl. Acad. Sci., 90:11478-11482 (1993B); Robinson et al., Vaccine, 11:957, (1993); Webster et al., Vaccine, 12:1495-1498 (1994)).

As is well known in the art, a large number of factors can influence the efficiency of expression of antigen genes and/or the immunogenicity of DNA vaccines. Examples of such factors

include the reproducibility of inoculation, construction of the plasmid vector, choice of the promoter used to drive antigen gene expression and stability of the inserted gene in the plasmid. Depending on their origin, promoters differ in tissue specificity and efficiency in initiating mRNA synthesis (Xiang et al., Virology, 209:564-579 (1994); Chapman et al., Nucle. Acids. Res., 19:3979-3986 (1991)). To date, most DNA vaccines in mammalian systems have relied upon viral promoters derived from cytomegalovirus (CMV). These have had good efficiency in both muscle and skin inoculation in a number of mammalian species. Another factor known to affect the immune response elicited by DNA immunization is the method of DNA delivery; parenteral routes can yield low rates of gene transfer and produce considerable variability of gene expression (Montgomery, 1993, supra). High-velocity inoculation of plasmids, using a gene-gun, enhanced the immune responses of mice (Fynan, 1993B, supra; Eisenbraun et al., DNA Cell Biol., 12: 791-797 (1993)), presumably because of a greater efficiency of DNA transfection and more effective antigen presentation by dendritic cells. Vectors containing the nucleic acid-based vaccine of the invention may also be introduced into the desired host by other methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), or a DNA vector transporter (see, e.g., Wu et al., J. Biol. Chem. 267:963-967 (1992); Wu and Wu, J. Biol. Chem. 263:14621-14624 (1988); Hartmut et al., Canadian Patent Application No. 2,012,311, filed Mar. 15, 1990), or any other known method or device.

Viral Vector Vaccines. As can be readily appreciated by one of ordinary skill in the art, nucleic acid vaccines of the present invention can also be incorporated into any recombinant virus and can be used to introduce a vaccine of the invention. Examples of suitable viruses that can act as recombinant viral hosts for vaccines, in addition to vaccinia, includes canarypox, adenovirus, and adeno-associated virus, as known in the art. Various genetically engineered virus hosts ("recombinant viruses") can be used to prepare viral vaccines for administration of nucleic acid encoding tumor antigens. Viral vaccines can promote a suitable immune response that targets activation of B lymphocytes, helper T lymphocytes, and cytotoxic T lymphocytes. Numerous virus species can be used as the recombinant virus hosts for the vaccines of the invention. A preferred recombinant virus for a viral vaccine is vaccinia virus (International Patent Publication WO 87/06262, Oct. 22, 1987, by Moss et al.; Cooney et al., Proc. Natl. Acad. Sci. USA 90:1882-6 (1993); Graham et al., J. Infect. Dis. 166:244-52 (1992); McElrath et al., J. Infect. Dis. 169:41-7 (1994)). In another embodiment, recombinant canarypox can be used (Pialoux et al., AIDS Res. Hum. Retroviruses 11:373-81 (1995), erratum in AIDS Res. Hum. Retroviruses 11:875 (1995); Andersson et al., J. Infect. Dis. 174:977-85 (1996); Fries et al.,

Vaccine 14:428-34 (1996); Gonczol et al., Vaccine 13:1080-5 (1995)). Another alternative is defective adenovirus or adenovirus (Gilardi-Hebenstreit et al., J. Gen. Virol. 71:2425-31 (1990); Prevec et al., J. Infect. Dis. 161:27-30 (1990); Lubeck et al., Proc. Natl. Acad. Sci. USA 86:6763-7 (1989); Xiang et al., Virology 219:220-7 (1996)). Other suitable viral vectors include retroviruses that are packaged in cells with amphotropic host range (see Miller, Human Gene Ther. 1:5-14 (1990); Ausubel et al., Current Protocols in Molecular Biology, sec. 9), and attenuated or defective DNA virus, such as but not limited to herpes simplex virus (HSV) (see, e.g., Kaplitt et al., Molec. Cell. Neurosci. 2:320-330 (1991)), papillomavirus, Epstein Barr virus (EBV), adeno-associated virus (AAV) (see, e.g., Samulski et al., J. Virol. 61:3096-3101 (1987); Samulski et al., J. Virol. 63:3822-3828 (1989)), US Patent Nos: 5990091, 5766599, 5756103, 6086890, 6274147, 05585254, 6140114, 5616326, 6099847, 6221136, 6086891, 5958425, 5744143, 5558860, 5266489, 5858368, 5795872, 5693530, 6020172, and the like, each entirely incorporated herein by reference.

Bi-functional plasmids for virus and DNA vaccines. Another aspect of the present invention concerns engineering of bi-functional plasmids that can serve as a DNA vaccine and a recombinant virus vector. Direct injection of the purified plasmid DNA, i.e., as a DNA vaccine, would elicit an immune response to the antigen expressed by the plasmid in test subjects. The plasmid would also be useful in live, recombinant viruses as immunization vehicles.

The bi-functional plasmid of the invention provides a heterologous gene, or an insertion site for a heterologous gene, under control of two different expression control sequences: an animal expression control sequence, and a viral expression control sequence. The term "under control" is used in its ordinary sense, i.e., operably or operatively associated with, in the sense that the expression control sequence, such as a promoter, provides for expression of a heterologous gene. In another embodiment, the animal expression control sequence is a mammalian promoter (avian promoters are also contemplated by the present invention); in a specific embodiment, the promoter is a late or early SV40 promoter, cytomegalovirus immediate early (CMV) promoter, a vaccinia virus early promoter, or a vaccinia virus late promoter, or any combination thereof. Subjects could be vaccinated with a multi-tiered regimen, with the bi-functional plasmid administered as DNA and, at a different time, but in any order, as a recombinant virus vaccine. The invention contemplates single or multiple administrations of the bi-functional plasmid as a DNA vaccine or as a recombinant virus vaccine, or both. This vaccination regimen may be complemented with administration of viral vaccines (infra), or may be used with additional vaccine vehicles.

As one of ordinary skill in the art can readily appreciate, the bi-functional plasmids of the invention can be used as nucleic acid vaccine vectors. Thus, by inserting at least 1 to about 50 different tumor genes into bi-functional plasmids, thus preparing a corresponding set of bi-functional plasmids useful as a nucleic acid vaccine can be prepared.

Active immunity elicited by vaccination with a tumor protein or proteins according to the present invention can prime or boost a cellular or humoral immune response. The tumor protein or proteins, or antigenic fragments thereof, can be prepared in an admixture with an adjuvant to prepare a vaccine.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response (Hood et al., Immunology, Second Ed., 1984, Benjamin/Cummings: Menlo Park, Calif., p. 384). Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol, and useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum. Selection of an adjuvant depends on the subject to be vaccinated. Preferably, a pharmaceutically acceptable adjuvant is used. For example, a vaccine for a human should avoid oil or hydrocarbon emulsion adjuvants, including complete and incomplete Freund's adjuvant. One example of an adjuvant suitable for use with humans is alum (alumina gel). In a specific embodiment, recombinant tumor protein is administered intramuscularly in alum. Alternatively, the recombinant tumor protein vaccine can be administered subcutaneously, intradermally, intraperitoneally, or via other acceptable vaccine administration routes.

Vaccine administration. According to the invention, immunization against tumors can be accomplished with a nucleic acid tumor/adjuvant vaccine of the invention alone, or in combination with a viral encoding tumor vaccine or a tumor protein vaccine, or both. In a specific embodiment, tumor nucleic acid or viral vaccine is provided intramuscularly (i.m.) to boost the immune response.

Each dose of vaccine may contain the same 1 to 50 nucleic acid sequences encoding the same

or different tumor proteins or portions thereof. Alteratively, the tumor sequences in subsequent vaccines may express different tumor genes or portions thereof. In yet another embodiment, the subsequent vaccines may have some tumor sequences in common, and others that are different, from the earlier vaccine. For example, the priming vaccine may contain nucleic acids expressing tumor proteins arbitrarily designated 1-2. A second (booster) vaccine may contain vaccines expressing tumor proteins 3-5 or 6-10, etc.

Tumor Vaccine Variants

As noted above, a tumor/adjuvant encoding nucleic acid for use in the vaccines of the invention can be obtained from different cancer or normal tumor patients or different geographically local isolates, or from geographically diverse isolates.

A tumor/adjuvant vaccine also includes nucleic acid encoding polypeptides having immunogenic activity elicited by an amino acid sequence of a tumor amino acid sequence as at least one epitope or antigenic determinant. Such amino acid sequences substantially correspond to at least one 10-200 amino acid fragment and/or consensus sequence of a known tumor antigen protein sequence, as described herein or as known in the art. Such a tumor antigen sequence can have overall homology or identity of at least 50% to a known tumor protein amino acid sequence, such as 50-99% homology, or any range or value therein, while eliciting an immunogenic response against at least one type of tumor protein, preferably including at least one pathologic form.

Percent homology can be determined, for example, by comparing sequence information using the GAP computer program, version 6.0. available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (J. Mol. Biol. 48:443 (1970)), as revised by Smith and Waterman (Adv. Appl. Math. 2:482 (1981)). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. The preferred default parameters for the GAP program include: (1) a unitary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted comparison matrix of Gribskov and Burgess, Nucl. Acids Res. 14:6745 (1986), as described by Schwartz and Dayhoff, eds., Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington, D.C. (1979), pp. 353-358; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

In another embodiment, a tumor/adjuvant vaccine of the present invention comprises a pathologic form of at least one tumor protein. Examples of such sequences are readily available from commercial and institutional tumor sequence databases, such as GENBANK, or other publically available databases. Substitutions or insertions of a tumor or cytokine to obtain an additional tumor or cytokine protein, encoded by a nucleic acid for use in a viral or nucleic acid vaccine of the present invention, can include substitutions or insertions of at least one amino acid residue (e.g., 1-25 amino acids). Alternatively, at least one amino acid (e.g., 1-25 amino acids) can be deleted from a tumor or cytokine sequence. Preferably, such substitutions, insertions or deletions are identified based on sequence determination of proteins obtained by nucleotide sequencing of at least one tumor or cytokine encoding nucleic acid from an individual.

Non-limiting examples of such substitutions, insertions or deletions preferably are made by the amplification of DNA or RNA sequences from tumor, which can be determined by routine experimentation to provide modified structural and functional properties of an protein or a tumor or cytokine. The tumor or cytokine protein seuquences so obtained preferably have different antigenic or adjuvant properties from the original tumor or cytokine. Such antigenic differences can be determined by suitable assays, e.g., by testing with a panel of monoclonal antibodies specific for tumor or cytokine proteins in an ELISA assay.

Any substitution, insertion or deletion can be used as long as the resulting tumor and cytokine proteins or antigenic determinants thereof elicits antibodies which bind to tumor proteins, but which tumor proteins have a different pattern than antibodies elicited by a second tumor protein. Each of the above substitutions, insertions or deletions can also include modified or unusual amino acids, e.g., as provided in 37 C.F.R. section 1.822(p)(2), which is entirely incorporated herein by reference.

The following present non-limiting examples of alternative nucleic acid sequences (recited as DNA sequences, but also including the corresponding RNA sequence (where U is substituted for T in the corresponding RNA sequence)) of tumor antigen proteins of tumors, as well as cytokine adjuvant nucleic acid sequences, that can be encoded by a nucleic acid according to present invention. Such nucleic acid vaccines can comprise at least one tumor antigen protein encoding nucleic acid and at least one cytokine adjuvant protein encoding nucleic acid, and can include linear or circular DNA or RNA, optionally further comprising additional regulatory sequences, such as but not limited to promoters, enhancers, selection, restriction sites, and the

like, as well known in the art. For amino acid sequences any suitable codon can be used for expression, preferably human preferred codons as well known in the art (see, e.g., Ausubel, supra, Appendices) and such sequences can be further modified, e.g., where specific antigenic sequences can be used.

SEQUENCE LISTING

PSA/KLK3 sequences

1. PSA (SEQ ID NO:1)

```
Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val
1               5                  10                  15

Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly Val Leu Val His
            20                  25                  30

Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg Asn Lys Ser Val
        35                  40                  45

Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu Asp Thr Gly Gln
    50                  55                  60

Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu Tyr Asp Met Ser
65                  70                  75                  80

Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp Ser Ser His Asp
                85                  90                  95

Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu Thr Asp Ala Val
            100                 105                 110

Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys
        115                 120                 125

Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe Leu Thr Pro
    130                 135                 140

Lys Lys Leu Gln Cys Val Asp Leu His Val Ile Ser Asn Asp Val Cys
145                 150                 155                 160

Ala Gln Val His Pro Gln Lys Val Thr Lys Phe Met Leu Cys Ala Gly
                165                 170                 175

Arg Trp Thr Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro
            180                 185                 190

Leu Val Cys Asn Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Ser Glu
        195                 200                 205
```

Pro Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr Thr Lys Val Val His
210 215 220

Tyr Arg Lys Trp Ile Lys Asp Thr Ile Val Ala Asn Pro
225 230 235

PSA 1: human PSA with introns (SEQ ID NO:2):

gtccgtgacg tggattggtg ctgcacccct catcctgtct cggattgtgg
gaggctggga 60
gtgcgagaag cattcccaac cctggcaggt gcttgtggcc tctcgtggca gggcagtctg 120
cggcggtgtt ctggtgcacc cccagtgggt cctcacagct gcccactgca tcaggaacaa 180
aagcgtgatc ttgctgggtc ggcacagcct gtttcatcct gaagacacag gccaggtatt 240
tcaggtcagc cacagcttcc cacacccgct ctacgatatg agcctcctga agaatcgatt 300
cctcaggcca ggtgatgact ccagccacga cctcatgctg ctccgcctgt cagagcctgc 360
cgagctcacg gatgctgtga aggtcatgga cctgcccacc caggagccag cactggggac 420
cacctgctac gcctcaggct ggggcagcat tgaaccagag gagttcttga ccccaaagaa 480
acttcagtgt gtggacctcc atgttatttc caatgacgtg tgtgcgcaag ttcaccctca 540
gaaggtgacc aagttcatgc tgtgtgctgg acgctggaca gggggcaaaa gcacctgctc 600
gggtgattct gggggcccac ttgtctgtaa tggtgtgctt caaggtatca cgtcatgggg 660
cagtgaacca tgtgccctgc ccgaaaggcc ttccctgtac accaaggtgg tgcattaccg 720
gaagtggatc aaggacacca tcgtggccaa cccctgagca cccctatcaa ccccctattg 780
tagtaaactt ggaaccttgg aaatgaccag gccaagactc aagcctcccc agttctactg 840
acctttgtcc ttaggtgtga ggtccagggt tgctaggaaa agaaatcagc agacacaggt 900
gtagaccaga gtgtttctta aatggtgtaa ttttgtcctc tctgtgtcct ggggaatact 960
ggccatgcct ggagacatat cactcaattt ctctgaggac acagatagga tggggtgtct 1020
gtgttatttg tggggtacag agatgaaaga ggggtgggat ccacactgag agagtggaga 1080
gtgacatgtg ctggacactg tccatgaagc actgagcaga agctggaggc acaacgcacc 1140
agacactcac agcaaggatg gagctgaaaa cataacccac tctgtcctgg aggcactggg 1200
aagcctagag aaggctgtga gccaaggagg gagggtcttc ctttggcatg ggatggggat 1260
gaagtaagga gagggactgg acccccctgga agctgattca ctatgggggg aggtgtattg 1320
aagtcctcca gacaaccctc agatttgatg atttcctagt agaactcaca gaaataaaga 1380
gctgttatac tgtg 1394

2. PSA 2: SEQ ID NO:1, comprising one or more or any combination of Thr40, Met112, and/or deletion of one or more of Tyr225, Arg226, Lys227, Trp228, Ile229, Lys230, Asp231, Thr232, Ile233, Val234, Ala235, Asn236, Pro237.

3. PSA 3: cDNA sequence with introns (SEQ ID NO:3):

aagtttccct tctcccagtc caagacccca aatcaccaca aaggacccaa tccccagact 61
caagatatgg tctgggcgct gtcttgtgtc tcctaccctg atccctgggt tcaactctgc 121
tcccagagca tgaagcctct ccaccagcac cagccaccaa cctgcaaacc tagggaagat 181
tgacagaatt cccagccttt cccagctccc cctgcccatg tcccaggact cccagccttg 241
gttctctgcc cccgtgtctt ttcaaaccca catcctaaat ccatctccta tccgagtccc 301
ccagttcctc ctgtcaaccc tgattccccct gatctagcac cccctctgca ggtgctgcac 361
ccctcatcct gtctcggatt gtgggaggct gggagtgcga gaagcattcc caaccctggc 421
aggtgcttgt agcctctcgt ggcagggcag tctgcggcgg tgttctggtg cacccccagt 481
gggtcctcac agctacccac tgcatcagga acaaaagcgt gatcttgctg ggtcggcaca 541
gcctgtttca tcctgaagac acaggccagg tatttcaggt cagccacagc ttcccacacc 601
cgctctacga tatgagcctc ctgaagaatc gattcctcag gccaggtgat gactccagcc 661
acgacctcat gctgctccgc ctgtcagagc ctgccgagct cacggatgct atgaaggtca 721
tggacctgcc cacccaggag ccagcactgg ggaccacctg ctacgcctca ggctggggca 781
gcattgaacc agaggagttc ttgaccccaa agaaacttca gtgtgtggac ctccatgtta 841
tttccaatga cgtgtgtgcg caagttcacc ctcagaaggt gaccaagttc atgctgtgtg 901
ctggacgctg gacaggggggc aaaagcacct gctcgggtga ttctgggggc ccacttgtct 961
gtaatggtgt gcttcaaggt atcacgtcat ggggcagtga accatgtgcc ctgcccgaaa 1021
ggccttccct gtacaccaag gtggtgcatt accggaagtg gatcaaggac accatcgtgg 1081

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ccaacccctg agcacccctа tcaactccct attgtagtaa acttggaacc ttggaaatga 1141
ccaggccaag actcaggcct ccccagttct actgaccttt gtccttaggt gtgaggtcca 1201
gggttgctag gaaaagaaat cagcagacac aggtgtagac cagagtgttt cttaaatggt 1261
gtaattttgt cctctctgtg tcctggggaa tactggccat gcctggagac atatcactca 1321
atttctctga ggacacagat aggatggggt gtctgtgtta tttgtggggt acagagatga 1381
aagaggggtg ggatccacac tgagagagtg gagagtgaca tgtgctggac actgtccatg 1441
aagcactgag cagaagctgg aggcacaacg caccagacac tcacagcaag gatggagctg 1501
aaaacataac ccactctgtc ctggaggcac tgggaagcct agagaaggct gtgaaccaag 1561
gagggagggt cttcctttgg catgggatgg ggatgaagta aggagaggga ctgacccct 1621
ggaagctgat tcactatggg gggaggtgta ttgaagtcct ccagacaacc ctcagatttg 1681
atgatttcct agtagaactc acagaaataa agagctgtta tactgtgaa
```

3. rhesus macaque PSA (SEQ ID NO:4):

```
Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val
1               5                  10                  15

Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly Val Leu Val His
            20                  25                  30

Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg Ser Asn Ser Val
        35                  40                  45

Ile Leu Leu Gly Arg His Asn Pro Tyr Tyr Pro Glu Asp Thr Gly Gln
    50                  55                  60

Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu Tyr Asn Met Ser
65                  70                  75                  80

Leu Leu Lys Asn Arg Tyr Leu Gly Pro Gly Asp Asp Ser Ser His Asp
                85                  90                  95

Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Ile Thr Asp Ala Val
            100                 105                 110

Gln Val Leu Asp Leu Pro Thr Trp Glu Pro Glu Leu Gly Thr Thr Cys
        115                 120                 125

Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu His Leu Thr Pro
    130                 135                 140

Lys Lys Leu Gln Cys Val Asp Leu His Ile Ile Ser Asn Asp Val Cys
145                 150                 155                 160

Ala Gln Val His Ser Gln Lys Val Thr Lys Phe Met Leu Cys Ala Gly
                165                 170                 175

Ser Trp Met Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro
            180                 185                 190

Leu Val Cys Asp Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Ser Gln
        195                 200                 205

Pro Cys Ala Leu Pro Arg Arg Pro Ser Leu Tyr Thr Lys Val Val Arg
    210                 215                 220
```

Tyr Arg Lys Trp Ile Gln Asp Thr Ile Met Ala Asn Pro
225 230 235

PSA 4: rhesus PSA : SEQ ID NO:4, comprising one or more or any combination of Thr40, Met112, and/or deletion of one or more of Tyr225, Arg226, Lys227, Trp228, Ile229, Gln230, Asp231, Thr232, Ile233, Met234, Ala235, Asn236, Pro237.

4. CTL epitopes from PSA

PSA antigen SEQ ID NO:5:
Phe Leu Thr Pro Lys Lys Leu Gln Cys Val
1 5 10

PSA antigen SEQ ID NO:6:
Lys Leu Gln Cys Val Asp Leu His Val
1 5

PSA antigen SEQ ID NO:7
Val Ile Ser Asn Asp Val Cys Ala Gln Val
1 5 10

PSA antigen SEQ ID NO:8
Val Leu Val His Pro Gln Trp Val Leu
1 5

PSA antigen SEQ ID NO:9
Gln Val His Pro Gln Lys Val Thr Lys
1 5

5. PSA antigen SEQ ID NO:10:

Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly Ala Ala Pro Leu
1 5 10 15

Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln
20 25 30

Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly
35 40 45

Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg
50 55 60

Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu
65 70 75 80

Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu
85 90 95

Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp

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                100                 105                 110

Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu
        115                 120                 125

Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala Leu
    130                 135                 140

Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu
145                 150                 155                 160

Phe Leu Thr Pro Lys Lys Leu Gln Cys Val Asp Leu His Val Ile Ser
                165                 170                 175

Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val Thr Lys Phe Met
            180                 185                 190

Leu Cys Ala Gly Arg Trp Thr Gly Gly Lys Ser Thr Cys Ser Trp Val
        195                 200                 205

Ile Leu Ile Thr Glu Leu Thr Met Pro Ala Leu Pro Met Val Leu His
    210                 215                 220

Gly Ser Leu Val Pro Trp Arg Gly Gly Val
225                 230
```

PSA cDNA (SEQ ID NO:11)

```
ggttgtcttc ctcaccctgt ccgtgacgtg gattggtgct gcacccctca tcctgtctcg 60
gattgtggga ggctgggagt gcgagaagca ttcccaaccc tggcaggtgc ttgtggcctc 120
tcgtggcagg gcagtctgcg gcggtgttct ggtgcacccc cagtgggtcc tcacagctgc 180
ccactgcatc aggaacaaaa gcgtgatctt gctgggtcgg cacagcctgt ttcatcctga 240
agacacaggc caggtatttc aggtcagcca cagcttccca cacccgctct acgatatgag 300
cctcctgaag aatcgattcc tcaggccagg tgatgactcc agccacgacc tcatgctgct 360
ccgcctgtca gagcctgccg agctcacgga tgctgtgaag gtcatggacc tgcccaccca 420
ggagccagca ctggggacca cctgctacgc ctcaggctgg ggcagcattg aaccagagga 480
gttcttgacc ccaaagaaac ttcagtgtgt ggacctccat gttatttcca atgacgtgtg 540
tgcgcaagtt caccctcaga aggtgaccaa gttcatgctg tgtgctggac gctggacagg 600
gggcaaaagc acctgctcgt gggtcattct gatcaccgaa ctgaccatgc cagccctgcc 660
gatggtcctc catggctccc tagtgccctg gagaggaggt gtctagtcag agagtagtcc 720
tggaaggtgg cctctgtgag gagccacggg gacagcatcc tgcagatggt cctggccctt 780
gtcccaccga cctgtctaca aggactgtcc tcgtggaccc tcccctctgc acaggagctg 840
gaccctgaag tcccttccct accggccagg actggagccc ctacccctct gttggaatcc 900
ctgcccacct tcttctggaa gtcggctctg gagacatttc tctcttcttc caaagctggg 960
aactgctatc tgttatctgc ctgtccaggt ctgaaagata ggattgccca ggcagaaact 1020
gggactgacc tatctcactc tctccctgct tttaccctta gggtgattct gggggcccac 1080
ttgtctgtaa tggtgtgctt caaggtatca cgtcatgggg cagtgaacca tgtgccctgc 1140
ccgaaaggcc ttccctgtac accaaggtgg tgcattaccg gaagtggatc aaggacacca 1200
tcgtggccaa cccctgagca cccctatcaa ctccctattg tagtaaactt ggaaccttgg 1260
aaatgaccag gccaagactc aagcctcccc agttctactg acctttgtcc ttaggtgtga 1320
ggtccagggt tgctaggaaa agaaatcagc agacacaggt gtagaccaga gtgtttctta 1380
aatggtgtaa ttttgtcctc tctgtgtcct ggggaatact ggccatgcct ggagacatat 1440
cactcaattt ctctgaggac acagatagga tgggttgtct gtgttatttg tggggtacag 1500
agatgaaaga ggggtgggga tccacactga gagagtggag agtgacatgt gctggacact 1560
gtccatgaag cactgagcag aagctggagg cacaacgcac cagacactca cagcaaggat 1620
ggagctgaaa acataaccca ctctgtcctg gagg                           1654
```

PSA ANTIGEN AA SEQ ID NO: 12

```
Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly Ala Ala Pro Leu
1               5                  10                  15

Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln
            20                  25                  30

Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly
        35                  40                  45

Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg
    50                  55                  60

Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu
65                  70                  75                  80

Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu
                85                  90                  95

Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp
            100                 105                 110

Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu
        115                 120                 125

Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala Leu
    130                 135                 140

Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu
145                 150                 155                 160

Cys Thr Pro Gly Pro Asp Gly Ala Ala Gly Ser Pro Asp Ala Trp Val
                165                 170                 175
```

PSA ANTIGEN DNA SEQ ID NO:13

```
ggttgtcttc ctcaccctgt ccgtgacgtg gattggtgct gcacccctca
tcctgtctcg 60
gattgtggga ggctgggagt gcgagaagca ttcccaaccc tggcaggtgc
ttgtggcctc 120
tcgtggcagg gcagtctgcg gcggtgttct ggtgcacccc cagtgggtcc
tcacagctgc 180
ccactgcatc aggaacaaaa gcgtgatctt gctgggtcgg cacagcctgt
ttcatcctga 240
agacacaggc caggtatttc aggtcagcca cagcttccca cacccgctct
acgatatgag 300
cctcctgaag aatcgattcc tcaggccagg tgatgactcc agccacgacc
tcatgctgct 360
ccgcctgtca gagcctgccg agctcacgga tgctgtgaag gtcatggacc
tgcccaccca 420
ggagccagca ctggggacca cctgctacgc ctcaggctgg ggcagcattg
aaccagagga 480
gtgtacgcct gggccagatg gtgcagccgg gagcccagat gcctgggtct
gagggaggag 540
```

gggacaggac tcctgggtct gagggaggag ggccaaggaa ccaggtgggg tccagcccac 600
aacagtgttt tttgcctggc ccgtagtctt gaccccaaag aaacttcagt gtgtggac 658

PSA ANTIGEN AA SEQ ID NO:14

Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly Ala Ala Pro Leu
1 5 10 15

Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln
20 25 30

Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly
35 40 45

Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg
50 55 60

Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu
65 70 75 80

Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu
85 90 95

Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp
100 105 110

Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu
115 120 125

Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala Leu
130 135 140

Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu
145 150 155 160

Cys Thr Pro Gly Pro Asp Gly Ala Ala Gly Ser Pro Asp Ala Trp Val
165 170 175

PSA ANTIGEN AA SEQ ID NO:15

Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val
1 5 10 15

Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly Val Leu Val His
20 25 30

Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg Lys Pro Gly Asp
35 40 45

Asp Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu
50 55 60

Leu Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala
65 70 75 80

Leu Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu
85 90 95

Glu Phe Leu Thr Pro Lys Lys Leu Gln Cys Val Asp Leu His Val Ile
100 105 110

Ser Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val Thr Lys Phe
115 120 125

Met Leu Cys Ala Gly Arg Trp Thr Gly Gly Lys Ser Thr Cys Ser Gly
130 135 140

Asp Ser Gly Gly Pro Leu Val Cys Asn Gly Val Leu Gln Gly Ile Thr
145 150 155 160

Ser Trp Gly Ser Glu Pro Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr
165 170 175

Thr Lys Val Val His Tyr Arg Lys Trp Ile Lys Asp Thr Ile Val Ala
180 185 190

Asn Pro

II. KLK2 sequences

KLK2 AA SEQ ID NO:16

Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val
1 5 10 15

Ala Val Tyr Ser His Gly Trp Ala His Cys Gly Gly Val Leu Val His
20 25 30

Pro Gln Trp Val Leu Thr Ala Ala His Cys Leu Lys Lys Asn Ser Gln
35 40 45

Val Trp Leu Gly Arg His Asn Leu Phe Glu Pro Glu Asp Thr Gly Gln
50 55 60

Arg Val Pro Val Ser His Ser Phe Pro His Pro Leu Tyr Asn Met Ser
65 70 75 80

Leu Leu Lys His Gln Ser Leu Arg Pro Asp Glu Asp Ser Ser His Asp
85 90 95

Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Lys Ile Thr Asp Val Val
100 105 110

Lys Val Leu Gly Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys
115 120 125

Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe Leu Arg Pro
130 135 140

Arg Ser Leu Gln Cys Val Ser Leu His Leu Leu Ser Asn Asp Met Cys
145 150 155 160

Ala Arg Ala Tyr Ser Glu Lys Val Thr Glu Phe Met Leu Cys Ala Gly

```
                165                 170                 175

Leu Trp Thr Gly Gly Lys Asp Thr Cys Gly Gly Asp Ser Gly Gly Pro
            180                 185                 190

Leu Val Cys Asn Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Pro Glu
        195                 200                 205

Pro Cys Ala Leu Pro Glu Lys Pro Ala Val Tyr Thr Lys Val Val His
    210                 215                 220

Tyr Arg Lys Trp Ile Lys Asp Thr Ile Ala Ala Asn Pro
225                 230                 235
```

KLK2 DNA SEQ ID NO:17

```
gctggatgtg gtggtgcatg cttgtggtct cagctatcct ggaggctgag acaggagaat 60
cggttgagtc tgggagttca aggctacagg gagctgcgat cacgccgctg cactccagcc 120
tgggaaacag agtgagactg tctcagaatt tttttaaaaa agaatcagtg atcatcccaa 180
cccctgttgc tgttcatcct gagcctgcct tctctggctt tgttccctag atcacatctc 240
catgatccat aggccctgcc caatctgacc tcacaccgtg ggaatgcctc cagactgatc 300
tagtatgtgt ggaacagcaa gtgctggctc tccctccccc tccacagctc tgggtgtggg 360
aggggttgt ccagcctcca gcagcatggg gagggccttg gtcagcatct aggtgccaac 420
agggcaaggg cggggtcctg gagaatgaag gctttatagg gctcctcagg gaggcccccc 480
agccccaaac tgcaccacct ggccgtggac acctgtgtca gcatgtggga cctggttctc 540
tccatcgcct tgtctgtggg gtgcactggt gagattgggg ggataaagga agggggggcgg 600
gttctgactc ttatgctgaa gccctttttcc tcccacccag tgccccagcc tcgtcccttc 660
agcccacagt tcagcccaga caatgtgccc ctgactcttc cacattgcaa tagtcctcat 720
gcccacacta ggtccccgct ccctcccact tacctcagac ctttctctcc attgcccagc 780
caaatccctg ctcccagctg ctttactaaa gagcaagttc ctaggcatct ctgtgtttct 840
ctttatgggg ttcaaaacct ttcaaggacc tctctccatg ccactggttc cttggaccct 900
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cgagcacccc caaccacaac ggccagttct ctgattccct aaatccgcac ccttttcaaa 1260
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tgggcactta acctttggtt tcttggaacc tcttaatctc ttagaaccca cagctgccac 1440
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cagatcactg aggataagct ggagccacaa tgcatgaggc acacacacag caaggatgac 6060
gctgtaaaca tagcccacgc tgtcctgggg gcactgggaa gcctagataa ggccgtgagc 6120
agaaagaagg ggaggatcc 6139

human KLK2 AA SEQ ID NO:18

```
Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val
1               5                  10                  15

Ala Val Tyr Ser His Gly Trp Ala His Cys Gly Gly Val Leu Val His
            20                  25                  30

Pro Gln Trp Val Leu Thr Ala Ala His Cys Leu Lys Lys Asn Ser Gln
        35                  40                  45

Val Trp Leu Gly Arg His Asn Leu Phe Glu Pro Glu Asp Thr Gly Gln
    50                  55                  60

Arg Val Pro Val Ser His Ser Phe Pro His Pro Leu Tyr Asn Met Ser
65                  70                  75                  80

Leu Leu Lys His Gln Ser Leu Arg Pro Asp Glu Asp Ser Ser His Asp
                85                  90                  95

Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Lys Ile Thr Asp Val Val
            100                 105                 110

Lys Val Leu Gly Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys
        115                 120                 125

Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu
    130                 135                 140
```

human KLK2 AA SEQ ID NO:19

```
Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val
1               5                  10                  15

Ala Val Tyr Ser His Gly Trp Ala His Cys Gly Gly Val Leu Val His
            20                  25                  30

Pro Gln Trp Val Leu Thr Ala Ala His Cys Leu Lys Lys Asn Ser Gln
        35                  40                  45

Val Trp Leu Gly Arg His Asn Leu Phe Glu Pro Glu Asp Thr Gly Gln
    50                  55                  60

Arg Val Pro Val Ser His Ser Phe Pro His Pro Leu Tyr Asn Met Ser
65                  70                  75                  80

Leu Leu Lys His Gln Ser Leu Arg Pro Asp Glu Asp Ser Ser His Asp
                85                  90                  95

Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Lys Ile Thr Asp Val Val
            100                 105                 110

Lys Val Leu Gly Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys
        115                 120                 125

Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe Leu Arg Pro
    130                 135                 140
```

```
Arg Ser Leu Gln Cys Val Ser Leu His Leu Leu Ser Asn Asp Met Cys
145                 150                 155                 160

Ala Arg Ala Tyr Ser Glu Lys Val Thr Glu Phe Met Leu Cys Ala Gly
                165                 170                 175

Leu Trp Thr Gly Gly Lys Asp Thr Cys Gly Val Ser His Pro Tyr Ser
            180                 185                 190

Gln His Leu Glu Gly Lys Gly
        195
```

III. MUC1 Sequences

human MUC1 AA: (SEQ ID NO:20)

```
Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Leu Thr
1               5                   10                  15

Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
            20                  25                  30

Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
        35                  40                  45

Thr Glu Lys Asn Ala Val Ser Met Thr Ser Ser Val Leu Ser Ser His
    50                  55                  60

Ser Pro Gly Ser Gly Ser Ser Thr Thr Gln Gly Gln Asp Val Thr Leu
65                  70                  75                  80

Ala Pro Ala Thr Glu Pro Ala Ser Gly Ser Ala Ala Thr Trp Gly Gln
                85                  90                  95

Asp Val Thr Ser Val Pro Val Thr Arg Pro Ala Leu Gly Ser Thr Thr
            100                 105                 110

Pro Pro Ala His Asp Val Thr Ser Ala Pro Asp Asn Lys Pro Ala Pro
        115                 120                 125

Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
    130                 135                 140

Arg Pro Pro Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
145                 150                 155                 160

Ala Pro Asp Thr Arg Pro Pro Pro Gly Ser Thr Ala Pro Ala Ala His
                165                 170                 175

Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala
            180                 185                 190

Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Asn Arg Pro Ala Leu
        195                 200                 205

Ala Ser Thr Ala Pro Pro Val His Asn Val Thr Ser Ala Ser Gly Ser
    210                 215                 220

Ala Ser Gly Ser Ala Ser Thr Leu Val His Asn Gly Thr Ser Ala Arg
225                 230                 235                 240
```

```
Ala Thr Thr Thr Pro Ala Ser Lys Ser Thr Pro Phe Ser Ile Pro Ser
                245                 250                 255

His His Ser Asp Thr Pro Thr Thr Leu Ala Ser His Ser Thr Lys Thr
            260                 265                 270

Asp Ala Ser Ser Thr His His Ser Thr Val Pro Pro Leu Thr Ser Ser
        275                 280                 285

Asn His Ser Thr Ser Pro Gln Leu Ser Thr Gly Val Ser Phe Phe Phe
    290                 295                 300

Leu Ser Phe His Ile Ser Asn Leu Gln Phe Asn Ser Ser Leu Glu Asp
305                 310                 315                 320

Pro Ser Thr Asp Tyr Tyr Gln Glu Leu Gln Arg Asp Ile Ser Glu Met
                325                 330                 335

Phe Leu Gln Ile Tyr Lys Gln Gly Gly Phe Leu Gly Leu Ser Asn Ile
            340                 345                 350

Lys Phe Arg Pro Gly Ser Val Val Val Gln Leu Thr Leu Ala Phe Arg
        355                 360                 365

Glu Gly Thr Ile Asn Val His Asp Val Glu Thr Gln Phe Asn Gln Tyr
    370                 375                 380

Lys Thr Glu Ala Ala Ser Arg Tyr Asn Leu Thr Ile Ser Asp Val Ser
385                 390                 395                 400

Val Ser Asp Val Pro Phe Pro Phe Ser Ala Gln Ser Gly Ala Gly Val
                405                 410                 415

Pro Gly Trp Gly Ile Ala Leu Leu Val Leu Val Cys Val Leu Val Ala
            420                 425                 430

Leu Ala Ile Val Tyr Leu Ile Ala Leu Ala Val Cys Gln Cys Arg Arg
        435                 440                 445

Lys Asn Tyr Gly Gln Leu Asp Ile Phe Pro Ala Arg Asp Thr Tyr His
    450                 455                 460

Pro Met Ser Glu Tyr Pro Thr Tyr His Thr His Gly Arg Tyr Val Pro
465                 470                 475                 480

Pro Ser Ser Thr Asp Arg Ser Pro Tyr Glu Lys Val Ser Ala Gly Asn
                485                 490                 495

Gly Gly Ser Ser Leu Ser Tyr Thr Asn Pro Ala Val Ala Ala Thr Ser
            500                 505                 510

Ala Asn Leu
        515
```

MUC1 DNA sequence: (SEQ ID NO:21)

```
gaattccctg gctgcttgaa tctgttctgc cccctcccca cccatttcac caccaccatg 60
acaccgggca cccagtctcc tttcttcctg ctgctgctcc tcacagtgct tacagttgtt 120
acaggttctg gtcatgcaag ctctacccca ggtggagaaa aggagacttc ggctacccag 180
agaagttcag tgcccagctc tactgagaag aatgctgtga gtatgaccag cagcgtactc 240
```

```
tccagccaca gccccggttc aggctcctcc accactcagg gacaggatgt cactctggcc 300
ccggccacgg aaccagcttc aggttcagct gccacctggg gacaggatgt cacctcggtc 360
ccagtcacca ggccagccct gggctccacc accccgccag cccacgatgt cacctcagcc 420
ccggacaaca agccagcccc gggctccacc gccccccccag cccacggtgt cacctcggcc 480
ccggacacca ggccgccccc gggctccacc gccccccccag cccacggtgt cacctcggcc 540
ccggacacca ggccgccccc gggctccacc gcgcccgcag cccacggtgt cacctcggcc 600
ccggacacca ggccggcccc gggctccacc gccccccccag cccatggtgt cacctcggcc 660
ccggacaaca ggcccgcctt ggcgtccacc gcccctccag tccacaatgt cacctcggcc 720
tcaggctctg catcaggctc agcttctact ctggtgcaca acggcacctc tgccagggct 780
accacaaccc cagccagcaa gagcactcca ttctcaattc ccagccacca ctctgatact 840
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ctgggagccc ccaccacaac acttcccagg catggaattc c                     1721
```

1. Complete coding sequence of MUC1 (genomic and protein translation, but does not include complete set of tandem repeats, probably in interest of space)

: (SEQ ID NO:22)

```
Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1               5                  10                  15

Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
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Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
        35                  40                  45

Thr Glu Lys Asn Ala Val Ser Met Thr Ser Ser Val Leu Ser Ser His
    50                  55                  60

Ser Pro Gly Ser Gly Ser Ser Thr Thr Gln Gly Gln Asp Val Thr Leu
65                  70                  75                  80

Ala Pro Ala Thr Glu Pro Ala Ser Gly Ser Ala Ala Thr Trp Gly Gln
                85                  90                  95

Asp Val Thr Ser Val Pro Val Thr Arg Pro Ala Leu Gly Ser Thr Thr
            100                 105                 110

Pro Pro Ala His Asp Val Thr Ser Ala Pro Asp Asn Lys Pro Ala Pro
        115                 120                 125

Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
    130                 135                 140
```

```
Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
145                 150                 155                 160

Ala Pro Asp Asn Arg Pro Ala Leu Gly Ser Thr Ala Pro Pro Val His
                165                 170                 175

Asn Val Thr Ser Ala Ser Gly Ser Ala Ser Gly Ser Ala Ser Thr Leu
            180                 185                 190

Val His Asn Gly Thr Ser Ala Arg Ala Thr Thr Thr Pro Ala Ser Lys
        195                 200                 205

Ser Thr Pro Phe Ser Ile Pro Ser His His Ser Asp Thr Pro Thr Thr
    210                 215                 220

Leu Ala Ser His Ser Thr Lys Thr Asp Ala Ser Ser Thr His His Ser
225                 230                 235                 240

Thr Val Pro Pro Leu Thr Ser Ser Asn His Ser Thr Ser Pro Gln Leu
                245                 250                 255

Ser Thr Gly Val Ser Phe Phe Phe Leu Ser Phe His Ile Ser Asn Leu
            260                 265                 270

Gln Phe Asn Ser Ser Leu Glu Asp Pro Ser Thr Asp Tyr Tyr Gln Glu
        275                 280                 285

Leu Gln Arg Asp Ile Ser Glu Met Phe Leu Gln Ile Tyr Lys Gln Gly
    290                 295                 300

Gly Phe Leu Gly Leu Ser Asn Ile Lys Phe Arg Pro Gly Ser Val Val
305                 310                 315                 320

Val Gln Leu Thr Leu Ala Phe Arg Glu Gly Thr Ile Asn Val His Asp
                325                 330                 335

Val Glu Thr Gln Phe Asn Gln Tyr Lys Thr Glu Ala Ala Ser Arg Tyr
            340                 345                 350

Asn Leu Thr Ile Ser Asp Val Ser Val Ser Asp Val Pro Phe Pro Phe
        355                 360                 365

Ser Ala Gln Ser Gly Ala Gly Val Pro Gly Trp Gly Ile Ala Leu Leu
    370                 375                 380

Val Leu Val Cys Val Leu Val Ala Leu Ala Ile Val Tyr Leu Ile Ala
385                 390                 395                 400

Leu Ala Val Cys Gln Cys Arg Arg Lys Asn Tyr Gly Gln Leu Asp Ile
                405                 410                 415

Phe Pro Ala Arg Asp Thr Tyr His Pro Met Ser Glu Tyr Pro Thr Tyr
            420                 425                 430

His Thr His Gly Arg Tyr Val Pro Pro Ser Ser Thr Asp Arg Ser Pro
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Tyr Glu Lys Val Ser Ala Gly Asn Gly Gly Ser Ser Leu Ser Tyr Thr
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Asn Pro Ala Val Ala Ala Thr Ser Ala Asn Leu
465 470 475

MUC-1 DNA SEQ ID NO:23

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2. 3′ end of MUC1 gene (contains exon 7, polyA signal and flanking region)

: (SEQ ID NO:24)

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cagatcatag ggcagactca ctgggggtgg ggggc 1835

3. 5' end of MUC1 gene (contains promoter and first ATG) : (SEQ ID NO:25)
First ATG is shown as last three residues below:

```
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gaactgtttt ctgaatacag gcggaactgc ttccgtcggc ctagaggcac gctgcgactg 1260
cgggacccaa gttccacgtg ctgccgcggc ctgggatagc ttcctccccc cgtgcactgc 1320
tgccgcacac acctcttggc tgtcgcgcat tacgcacctc acgtgtgctt ttgccccccg 1380
ctacgtgcct acctgtcccc aataccactc tgctccccaa aggatagttc tgtgtccgta 1440
aatcccattc tgtcacccca cctactctct gccccccccc ttttttgttt gagacggagc 1500
tttgctctgt cgcccaggct ggagtgcaat ggcgcgatct cggctcactg caacctccgc 1560
ctcccgggtt caagcgattc tcctgcctca gcctcctgag tagctggggt tacagcgccc 1620
gccaccacgc tcggctaatt tttgtagttt ttagtagaga cgaggtttca ccatcttggc 1680
caggctggtc ttgaaccccc gaccttgtga tccactcgcc tcggccttcc aaagtgttgg 1740
gattacgggc gtgacgaccg tgccacgcat ctgcctctta agtacataac ggcccacaca 1800
gaacgtgtcc aactcccccg cccacgttcc aacgtcctct cccacatacc tcggtgcccc 1860
ttccacatac ctcaggaccc cacccgctta gctccatttc ctccagacgc caccaccacg 1920
cgtcccggag tgccccctcc taaagctccc agccgtccac catgctgtgc gttcctccct 1980
ccctggccac ggcagtgacc cttctctccc gggccctgct tccctctcgc gggctctgct 2040
gcctcactta ggcagcgctg cccttactcc tctccgcccg gtccgagcgg cccctcagct 2100
tcggcgccca gccccgcaag gctcccggtg accactagag ggcgggagga gctcctggcc 2160
agtggtggag agtggcaagg aaggacccta gggttcatcg gagcccaggt ttactccctt 2220
aagtggaaat ttcttccccc actcctcctt ggctttctcc aaggagggaa cccaggctgc 2280
tggaaagtcc ggctggggcg gggactgtgg gttcagggga gaacggggtg tggaacggga 2340
cagggagcgg ttagaagggt ggggctattc cgggaagtgg tgggggggagg gagcccaaaa 2400
ctagcaccta gtccactcat tatccagccc tcttatttct cggccgctct gcttcagtgg 2460
acccgggggag ggcggggaag tggagtggga gacctagggg tgggcttccc gaccttgctg 2520
tacaggacct cgacctagct ggctttgttc cccatcccca cgttagttgt tgccctgagg 2580
ctaaaactag agcccagggg ccccaagttc cagactgccc ctccccccctc ccccggagcc 2640
agggagtggt tggtgaaagg gggaggccag ctggagaaca aacgggtagt caggggggttg 2700
agcgattaga gcccttgtac cctacccagg aatggttggg gaggaggagg aagaggtagg 2760
aggtagggga gggggcgggg ttttgtcacc tgtcacctgc tcgctgtgcc tagggcgggc 2820
gggcggggag tgggggggacc ggtataaagc ggtaggcgcc tgtgcccgct ccacctctca 2880
agcagccagc gcctgcctga atctgttctg ccccctcccc acccatttca ccaccaccat 2940
g                                                                  2941
```

4. Differentially spliced forms of MUC1

a. cDNA sequence of "MUC1 seq" : (SEQ ID NO:26)

```
Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1               5                  10                  15

Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
            20                  25                  30

Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
        35                  40                  45

Thr Glu Lys Asn Ala Val Ser Met Thr Ser Ser Val Leu Ser Ser His
    50                  55                  60

Ser Pro Gly Ser Gly Ser Ser Thr Thr Gln Gly Gln Asp Val Thr Leu
65                  70                  75                  80

Ala Pro Ala Thr Glu Pro Ala Ser Gly Ser Ala Ala Thr Trp Gly Gln
                85                  90                  95

Asp Val Thr Ser Val Pro Val Thr Arg Pro Ala Leu Gly Ser Thr Thr
            100                 105                 110

Pro Pro Ala His Asp Val Thr Ser Ala Pro Asp Asn Lys Pro Ala Pro
        115                 120                 125

Gly Ser Thr Ala Pro Pro Ala Gln Gly Val Thr Ser Ala Pro Glu Thr
    130                 135                 140

Arg Pro Pro Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
145                 150                 155                 160

Ala Pro Asp Asn Arg Pro Ala Leu Ala Ser Thr Ala Pro Pro Val His
                165                 170                 175

Asn Val Thr Ser Ala Ser Gly Ser Ala Ser Gly Ser Ala Ser Thr Leu
            180                 185                 190

Val His Asn Gly Thr Ser Ala Arg Ala Thr Thr Thr Pro Ala Ser Lys
        195                 200                 205

Ser Thr Pro Phe Ser Ile Pro Ser His His Ser Asp Thr Pro Thr Thr
    210                 215                 220

Leu Ala Ser His Ser Thr Lys Thr Asp Ala Ser Ser Thr His His Ser
225                 230                 235                 240

Thr Val Pro Pro Leu Thr Ser Ser Asn His Ser Thr Ser Pro Gln Leu
                245                 250                 255

Ser Thr Gly Val Ser Phe Phe Phe Leu Ser Phe His Ile Ser Asn Leu
            260                 265                 270

Gln Phe Asn Ser Ser Leu Glu Asp Pro Ser Thr Asp Tyr Tyr Gln Glu
        275                 280                 285

Leu Gln Arg Asp Ile Ser Glu Met Val Ser Ile Gly Leu Ser Phe Pro
    290                 295                 300

Met Leu Pro
```

305

: (SEQ ID NO:27)

gagctcctgg ccagtggtgg agagtggcaa ggaaggaccc tagggttcat cggagcccag 60
gtttactccc ttaagtggaa atttcttccc ccactcccct ccttggcttt ctccaaggag 120
ggaaccccag gctgctggaa agtccggctg gggcggggac tgtgggtttc agggtagaac 180
tgcgtgtgga acgggacagg gagcggttag aagggtgggg ctattccggg aagtggtggt 240
gggggggaggg agcccaaaac tagcacctag tccactcatt atccagccct cttatttctc 300
ggccgcctct gcttcagtgg acccggggag ggcggggaag tggagtggga gacctagggg 360
tgggcttccc gaccttgctg tacaggacct cgacctagct ggctttgttc cccatcccca 420
gttagttgtt gccctgaggc taaaactaga gcccaggggc cccaagttcc agactgcccc 480
tccccccctcc cccggagcca gggagtggtt ggtgaaaggg ggaggccagc tggagaagaa 540
acgggtagtc aggggttgca gcattagagc ccttgtagcc ctagcccagg aatggttgga 600
gagagaagag tagagtaggg agggggggttt gtcacctgtc acctgctcgg ctgtgcctag 660
ggcgggcggg ggggagtggg gggaccggta taaagcggta ggcgcctgtg cccgctccac 720
ctctcaagca gccagcgcct gcctgaatct gttctgcccc ctccccaccc atttcaccac 780
caccatgaca ccgggcaccc agtctccttt cttcctgctg ctgctcctca cagtgcttac 840
aggtgagggg cacgaggtgg ggagtgggct gccctgctta ggtggtcttc gtggtctttc 900
tgtgggtttt gctccctggc agatggcacc agaagttaag gtaagaattg cagacagagg 960
ctgccctgtc tgtgccagaa ggagggagag gctaaggaca ggctgagaag agttgccccc 1020
aaccctgaga gtgggtacca ggggcaagca aatgtcctgt agagaagtct agggggaaga 1080
gagtagggag agggaaggct taagagggga agaaatgcag gggccatgag ccaaggccta 1140
tgggcagaga gaaggaggct gctgcaggaa ggaggcggcc aacccagggg ttactgaggc 1200
tgcccactcc ccagtcctcc tggtattatt tctctggtgg ccaggcttat attttcttct 1260
tgctcttatt tttccttcat aaagacccaa ccctatgact ttaacttctt acagctacca 1320
cagcccctgg gcccgcaaca gttgttacag gttctggtca tgcaagctct accccaggtg 1380
gagaaaagga gacttcggct acccagagaa gttcagtgcc cagctctact gagaagaatg 1440
ctgtgagtat gaccagcagc gtactctcca gccacagccc cggttcaggc tcctccacca 1500
ctcagggaca ggatgtcact ctggccccgg ccacggaacc agcttcaggt tcagctgcca 1560
cctggggaca ggatgtcacc tcggtcccag tcaccaggcc agccctgggc tccaccaccc 1620
cgccagccca cgatgtcacc tcagccccgg acaacaagcc agccccgggc tccaccgccc 1680
ccccagccca gggtgtcacc tcggccccgg agaccaggcc gccccgggc tccaccgccc 1740
ccccagccca tggtgtcacc tcggcgccgg acaacaggcc cgccttggcg tccaccgccc 1800
ctccagtcca caatgtcacc tcggcctcag gctctgcatc aggctcagct tctactctgg 1860
tgcacaacgg cacctctgcc agggctacca caaccccagc cagcaagagc actccattct 1920
caattcccag ccaccactct gatactccta ccacccttgc cagccatagc accaagactg 1980
atgccagtag cactcaccat agcacggtac ctcctctcac ctcctccaat cacagcactt 2040
ctccccagtt gtctactggg gtctctttct ttttcctgtc ttttcacatt tcaaacctcc 2100
agtttaattc ctctctggaa gatcccagca ccgactacta ccaagagctg cagagagaca 2160
tttctgaaat ggtgagtatc ggcctttcct tccccatgct cccctgaagc agccatcaga 2220
actgtccaca ccctttgcat caagcctgag tcctttccct ctcaccccag tttttgcaga 2280
tttataaaca agggggtttt ctgggcctct ccaatattaa gttcaggtac agttctgggt 2340
gtggacccag tgtggtggtt ggaggggtgg gtggtggtca tgagccgtag ggagggactg 2400
gtgcacttaa ggttggggga agagtgctga gccagagctg ggacccgtgg ctgaagtgcc 2460
catttccctg tgaccaggcc aggatctgtg gtggtacaat tgactctggc cttccgagaa 2520
ggtaccatca atgtccacga cgtggagaca cagttcaatc agtataaaac ggaagcagcc 2580
tctcgatata acctgacgat ctcaagacgt cagcggtgag gctacttccc tgctgcagcc 2640
agcaccatgc cggggccccct ctccttccag tgtctgggtc cccgctcttt ccttagtgct 2700
ggcagcggga ggggcgcctc ctctgggaga ctgccctgac cactgctttt ccttttagtg 2760
agtgatgtgc catttccttt ctctgaccag tctggggctg gggtgccagg ctggggcatc 2820
gcgctgctgg tgctggtctg tgttctggtt gcgctggcca ttgtctatct cattgccttg 2880
gtgagtgcag tccctggccc tgatcagagc cccccggtag aaggcactcc atggcctgcc 2940
ataacctcct atctccccag gctgtctgtc agtgccgccg aaagaactac gggcagctgg 3000
acatctttcc agcccgggat acctaccatc ctatgagcga gtaccccacc taccacaccc 3060
atgggcgcta tgtgccccta gcagtaccga tcgtagcccc tatgagaagg tgagattggg 3120
ccccacaggc aggggaagca gagggtttgg ctgggcaagg attctgaagg gggtacttgg 3180
aaaacccaaa gagcttggaa gaggtgagaa gtggcgtgaa gtgagcaggg gagggctggc 3240
aaggatgagg ggcagaggtc agaggagttt tgggggacag gcctgggagg agactatgga 3300

agaaaggggc ccctcaaaag ggagtgcccc actgccagaa ttc 3343

b. DNA sequence of MUC1Y: (SEQ ID NO:28)

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1 5 10 15

Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
20 25 30

Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
35 40 45

Thr Glu Lys Asn Ala Phe Asn Ser Ser Leu Glu Asp Pro Ser Thr Asp
50 55 60

Tyr Tyr Gln Glu Leu Gln Arg Asp Ile Ser Glu Met Phe Leu Gln Ile
65 70 75 80

Tyr Lys Gln Gly Gly Phe Leu Gly Leu Ser Asn Ile Lys Phe Arg Pro
85 90 95

Gly Ser Val Val Val Gln Leu Thr Leu Ala Phe Arg Glu Gly Thr Ile
100 105 110

Asn Val His Asp Val Glu Thr Gln Phe Asn Gln Tyr Lys Thr Glu Ala
115 120 125

Ala Ser Arg Tyr Asn Leu Thr Ile Ser Asp Val Ser Val Ser Asp Val
130 135 140

Pro Phe Pro Phe Ser Ala Gln Ser Gly Ala Gly Val Pro Gly Trp Gly
145 150 155 160

Ile Ala Leu Leu Val Leu Val Cys Val Leu Val Ala Leu Ala Ile Val
165 170 175

Tyr Leu Ile Ala Leu Ala Val Cys Gln Cys Arg Arg Lys Asn Tyr Gly
180 185 190

Gln Leu Asp Ile Phe Pro Ala Arg Asp Thr Tyr His Pro Met Ser Glu
195 200 205

Tyr Pro Thr Tyr His Thr His Gly Arg Tyr Val Pro Pro Ser Ser Thr
210 215 220

Asp Arg Ser Pro Tyr Glu Lys Val Ser Ala Gly Asn Gly Gly Ser Ser
225 230 235 240

Leu Ser Tyr Thr Asn Pro Ala Val Ala Ala Thr Ser Ala Asn Leu
245 250 255

: (SEQ ID NO:29)
atgacaccgg gcacccagtc tcctttcttc ctgctgctgc tcctcacagt gcttacagtt 60
gttacaggtt ctggtcatgc aagctctacc ccaggtggag aaaaggagac ttcggctacc 120
cagagaagtt cagtgcccag ctctactgag aagaatgctt ttaattcctc tctggaagat 180
cccagcaccg actactacca agagctgcag agagacattt ctgaaatgtt tttgcagatt 240

tataaacaag ggggttttct gggcctctcc aatattaagt tcaggccagg atctgtggtg 300
gtacaattga ctctggcctt ccgagaaggt accatcaatg tccacgacgt ggagacacag 360
ttcaatcagt ataaaacgga agcagcctct cgatataacc tgacgatctc agacgtcagc 420
gtgagtgatg tgccatttcc tttctctgcc cagtctgggg ctggggtgcc aggctggggc 480
atcgcgctgc tggtgctggt ctgtgttctg gttgcgctgg ccattgtcta tctcattgcc 540
ttggctgtct gtcagtgccg ccgaaagaac tacgggcagc tggacatctt tccagcccgg 600
gatacctacc atcctatgag cgagtacccc acctaccaca cccatgggcg ctatgtgccc 660
cctagcagta ccgatcgtag cccctatgag aaggtttctg caggtaatgg tggcagcagc 720
ctctcttaca caaacccagc agtggcagcc acttctgcca acttgtag 768

c. MUC-1 AA:: (SEQ ID NO:30)

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1 5 10 15

Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
20 25 30

Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
35 40 45

Thr Glu Lys Asn Ala Leu Ser Thr Gly Val Ser Phe Phe Phe Leu Ser
50 55 60

Phe His Ile Ser Asn Leu Gln Phe Asn Ser Ser Leu Glu Asp Pro Ser
65 70 75 80

Thr Asp Tyr Tyr Gln Glu Leu Gln Arg Asp Ile Ser Glu Met Ala Val
85 90 95

Cys Gln Cys Arg Arg Lys Asn Tyr Gly Leu Leu Asp Ile Phe Pro Ala
100 105 110

Arg Asp Thr Tyr His Pro Met Ser Glu Tyr Pro Thr Tyr His Thr His
115 120 125

Gly Arg Tyr Val Pro Pro Ser Ser Thr Asp Arg Ser Pro Tyr Glu Lys
130 135 140

Val Ser Ala Gly Asn Gly Gly Ser Ser Leu Ser Tyr Thr Asn Pro Ala
145 150 155 160

Val Ala Ala Thr Ser Ala Asn Leu
165

: (SEQ ID NO:31)

ctccccaccc atttcaccac caccatgaca ccgggcaccc agtctccttt cttcctgctg 60
ctgctcctca cagtgcttac agttgttaca ggttctggtc atgcaagctc taccccaggt 120
ggagaaaagg agacttcggc tacccagaga agttcagtgc ccagctctac tgagaagaat 180
gctttgtcta ctggggtctc tttctttttc ctgtcttttc acatttcaaa cctccagttt 240
aattcctctc tggaagatcc cagcaccgac tactaccaag agctgcagag agacatttct 300
gaaatggctg tctgtcagtg ccgccgaaag aactacgggc tgctggacat ctttccagcc 360
cgggatacct accatcctat gagcgagtac cccacctacc acacccatgg gcgctatgtg 420
ccccctagca gtaccgatcg tagcccctat gagaaggttt ctgcaggtaa tggtggcagc 480
agcctctctt acacaaaccc agcagtggca gccacttctg ccaacttgta ggggcacgtc 540
gcc 543

d. cDNA of a variant of "MUC1Y": (SEQ ID NO:32)

```
Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1               5                  10                  15

Val Leu Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly Gly Glu Lys
            20                  25                  30

Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser Thr Glu Lys
        35                  40                  45

Asn Ala Phe Asn Ser Ser Leu Glu Asp Pro Ser Thr Asp Tyr Tyr Gln
    50                  55                  60

Glu Leu Gln Arg Asp Ile Ser Glu Met Phe Leu Gln Ile Tyr Lys Gln
65                  70                  75                  80

Gly Gly Phe Leu Gly Leu Ser Asn Ile Lys Phe Arg Pro Gly Ser Val
                85                  90                  95

Val Val Gln Leu Thr Leu Ala Phe Arg Glu Gly Thr Ile Asn Val His
            100                 105                 110

Asp Met Glu Thr Gln Phe Asn Gln Tyr Lys Thr Glu Ala Ala Ser Arg
        115                 120                 125

Tyr Asn Leu Thr Ile Ser Asp Val Ser Val Ser Asp Val Pro Phe Pro
    130                 135                 140

Phe Ser Ala Gln Ser Gly Ala Gly Val Pro Gly Trp Gly Ile Ala Leu
145                 150                 155                 160

Leu Val Leu Val Cys Val Leu Val Ala Leu Ala Ile Val Tyr Leu Ile
                165                 170                 175

Ala Leu Ala Val Cys Gln Cys Arg Arg Lys Asn Tyr Gly Gln Leu Asp
            180                 185                 190

Ile Phe Pro Ala Arg Asp Thr Tyr His Pro Met Ser Glu Tyr Pro Thr
        195                 200                 205

Tyr His Thr His Gly Arg Tyr Val Pro Pro Ser Ser Thr Asp Arg Ser
    210                 215                 220

Pro Tyr Glu Lys Val Ser Ala Gly Asn Gly Gly Ser Ser Leu Ser Tyr
225                 230                 235                 140

Thr Asn Pro Ala Val Ala Ala Thr Ser Ala Asn Leu
                245                 250
```

: (SEQ ID NO:33)

```
atgacaccgg gcacccagtc tcctttcttc ctgctgctgc tcctcacagt gcttacaggt 60
tctggtcatg caagctctac cccaggtgga gaaaaggaga cttcggctac ccagagaagt 120
tcagtgccca gctctactga gaagaatgct tttaattcct ctctggaaga tcccagcacc 180
gactactacc aagagctgca gagagacatt tctgaaatgt ttttgcagat ttataaacaa 240
gggggttttc tgggcctctc caatattaag ttcaggccag gatctgtggt ggtacaattg 300
actctggcct tccgagaagg taccatcaat gtccacgaca tggagacaca gttcaatcag 360
tataaaacgg aagcagcctc tcgatataac ctgacgatct cagacgtcag cgtgagtgat 420
gtgccatttc ctttctctgc ccagtctggg gctggggtgc caggctgggg catcgcgctg 480
ctggtgctgg tctgtgttct ggttgcgctg gccattgtct atctcattgc cttggctgtc 540
```

tgtcagtgcc gccgaaagaa ctacgggcag ctggacatct ttccagcccg ggatacctac 600
catcctatga gcgagtaccc cacctaccac acccatgggc gctatgtgcc ccctagcagt 660
accgatcgta gcccctatga gaaggtttct gcaggtaatg gtggcagcag cctctcttac 720
acaaacccag cagtggcagc cacttctgcc aacttgtag 759

Reference: no published reference, only the database information

e. MUC1X or MUC1Z partial cDNA sequence: : (SEQ ID NO:34)

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1 5 10 15

Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
20 25 30

Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
35 40 45

Thr Glu Lys Asn Ala Leu Ser Thr Gly Val Ser Phe Phe Phe Leu Ser
50 55 60

Phe His Ile Ser Asn Leu Gln Phe Asn Ser Ser Leu Glu
65 70 75

f. S81781, cDNA: (SEQ ID NO:35)

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Leu Thr
1 5 10 15

Val Leu Thr Ala Thr Thr Ala Pro Lys Pro Ala Thr Val Val Thr Gly
20 25 30

Ser Gly His Ala Ser Ser Thr Pro Gly Gly Glu Lys Glu Thr Ser Ala
35 40 45

Thr Gln Arg Ser Ser Val Pro Ser Ser Thr Glu Lys Asn Ala Val Ser
50 55 60

Met Thr Ser Ser Val Leu Ser Ser His Ser Pro Gly Ser Gly Ser Ser
65 70 75 80

Thr Thr Gln Gly Gln Asp Val Thr Leu Ala Pro Ala Thr Glu Pro Ala
85 90 95

Ser Gly Ser Ala Ala Thr Trp Gly Gln Asp Val Thr Ser
100 105

: (SEQ ID NO:36)

accaccacca tgacaccggg cacccagtct cctttcttcc tgctgctgct cctcacagtg 60
cttacagcta ccacagcccc taaacccgca acagttgtta caggttctgg tcatgcaagc 120
tctaccccag gtggagaaaa ggagacttcg gctacccaga gaagttcagt gcccagctct 180
actgagaaga atgctgtgag tatgaccagc agcgtactct ccagccacag ccccggttca 240
ggctcctcca ccactcaggg acaggatgtc actctggccc cggccacgga accagcttca 300
ggttcagctg ccacctgggg acaggatgtc acctcg 336

Reference: Int. J. Cancer 66 (1), 55-59 (1996)

g. M32738, partial cDNA of MUC1 splice variant A: : (SEQ ID NO:37)

```
Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1               5                  10                  15

Val Leu Thr Ala Thr Thr Ala Pro Lys Pro Ala Thr Val Val Thr Gly
            20                  25                  30

Ser Gly His Ala Ser Ser Thr Pro Gly Gly Glu Lys Glu Thr Ser Ala
        35                  40                  45

Thr Gln Arg Ser Ser Val Pro Ser Ser Thr Glu Lys Asn Ala Val Ser
    50                  55                  60

Met Thr Ser Ser Val Leu Ser Ser His Ser Pro Gly Ser Gly Ser Ser
65                  70                  75                  80

Thr Thr Gln Gly Gln Asp Val Thr Leu Ala Pro Ala Thr Glu Pro Ala
                85                  90                  95

Ser Gly Ser Ala Ala Thr Trp Gly Gln Asp Val Thr Ser Val Pro Val
            100                 105                 110

Thr Arg Pro Ala Leu Gly Ser Thr Thr Pro Pro Ala His Asp Val Thr
        115                 120                 125

Ser Ala Pro Asp Asn Lys Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala
    130                 135                 140

His Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala
145                 150                 155
```

: (SEQ ID NO:38)

```
gcgcctgcct gaatctgttc tgccccctcc ccacccattt caccaccacc atgacaccgg 60
gcacccagtc tcctttcttc ctgctgctgc tcctcacagt gcttacagct accacagccc 120
ctaaacccgc aacagttgtt acaggttctg gtcatgcaag ctctacccca ggtggagaaa 180
aggagacttc ggctacccag agaagttcag tgcccagctc tactgagaag aatgctgtga 240
gtatgaccag cagcgtactc tccagccaca gccccggttc aggctcctcc accactcagg 300
gacaggatgt cactctggcc ccggccacgg aaccagcttc aggttcagct gccacctggg 360
gacaggatgt cacctcggtc ccagtcacca ggccagccct gggctccacc accccgccag 420
cccacgatgt cacctcagcc ccggacaaca agccagcccc gggctccacc gccccccag 480
cccacggtgt cacctcggcc ccggacacca ggccggcc                        518
```

Reference: J. Biol. Chem. 265, 5573-5578 (1990)

h. Z17324, partial cDNA of MUC1 splice variant C: (SEQ ID NO:39)

```
Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1               5                  10                  15

Val Leu Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly Gly Glu Lys
            20                  25                  30

Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro
        35                  40
```

: (SEQ ID NO:40)
ccgctccacc tctcaagcag ccagcgcctg cctgaatctg ttctgccccc tccccaccca 60
tttcaccacc accatgacac cgggcaccca gtctcctttc ttcctgctgc tgctcctcac 120
agtgcttaca ggttctggtc atgcaagctc taccccaggt ggagaaaagg agacttcggc 180
tacccagaga agttcagtgc ccag 204

Reference: no literature reference, a direct submission to the database

i. Z17325, partial cDNA of MUC1 splice variant D

: (SEQ ID NO:41)
Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Leu Thr
1 5 10 15

Val Leu Thr Gly Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser
20 25 30

Val Pro

: (SEQ ID NO:42)
ccgctccacc tctcaagcag ccagcgcctg cctgaatctg ttctgccccc tccccaccca 60
tttcaccacc accatgacac cgggcaccca gtctcctttc ttcctgctgc tgctcctcac 120
agtgcttaca ggtggagaaa aggagacttc ggctacccag agaagttcag tgcccag 177

5. CTL epitopes of MUC1: : (SEQ ID NO:43)

Ser Thr Ala Pro Pro Val His Asn Val
1 5

Reference: Blood 93:4309-4317, 1999

: (SEQ ID NO:44)
Leu Leu Leu Leu Thr Val Leu Thr Val
1 5

Reference: Blood 93:4309-4317, 1999

: (SEQ ID NO:45)
Ser Thr Ala Pro Pro Ala His Gly Val
1 5

Reference J Immunology 155:4766-4774, 1995; J Immunology 159:5211-5218, 1997

: (SEQ ID NO:46)

Ala Pro Asp Thr Arg Pro Ala
1 5

Reference J Immunology 159:5211-5218, 1997

6. CD4 T helper epitopes of MUC1

: (SEQ ID NO:47)
Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr
1 5 10

for HLA DR3 Reference: Cancer Research 58: 5066-5070, 1998

IV. Sequences for DNA vaccine vectors:

1. HCMV promoter/enhancer; K01484 Mark Stinski U Iowa

490 bp of promoter sequence, to transcriptional start
: (SEQ ID NO:48
ggcgaccgcc cagcgacccc cgcccgttga cgtcaatagt gacgtatgtt cccatagtaa 60
cgccaatagg gactttccat tgacgtcaat gggtggagta tttacggtaa actgcccact 120
tggcagtaca tcaagtgtat catatgccaa gtccgccccc tattgacgtc aatgacggta 180
aatggcccgc ctagcattat gcccagtaca tgaccttacg ggagtttcct acttggcagt 240
acatctacgt attagtcatc gctattacca tggtgatgcg gttttggcag tacaccaatg 300
ggcgtggata gcggtttgac tcacggggat ttccaagtct ccaccccatt gacgtcaatg 360
ggagtttgtt ttggcaccaa aatcaacggg actttccaaa atgtcgtaat aaccccgccc 420
cgttgacgca aatgggcggt aggcgtgtac ggtgggaggt ctatatagca gagctcgttt 480
agtgaaccgt cagatcgcct ggagacgcca tccacgctgt tttgacctcc atagaagaca 540
ccgggaccga tccagcctcc gcggccggga acggtgcatt ggaacgcgga ttccccgtgc 600
caagagtgac gtaagt 616

Reference: J. Virol. 49, 190-199 (1984); Proc. Natl. Acad. Sci. U.S.A. 81, 659-663 (1984)

2. HCMV promoter/enhancer; K03104
: (SEQ ID NO:49)
737bp of promoter sequence, to +193bp; includes exon 1 and part of intron A

aatcaatatt ggccattagc catattattc attggttata tagcataaat caatattggc 60
tattggccat tgcatacgtt gtatccatat cataatatgt acatttatat tggctcatgt 120
ccaacattac cgccatgttg acattgatta ttgactagtt attaatagta atcaattacg 180
gggtcattag ttcatagccc atatatggag ttccgcgtta cataacttac ggtaaatggc 240
ccgcctggct gaccgcccaa cgacccccgc ccattgacgt caataatgac gtatgttccc 300
atagtaacgc caatagggac tttccattga cgtcaatggg tggagtattt acggtaaact 360
gcccacttgg cagtacatca agtgtatcat atgccaagta cgccccctat tgacgtcaat 420
gacggtaaat ggcccgcctg gcattatgcc cagtacatga ccttatggga ctttcctact 480
tggcagtaca tctacgtatt agtcatcgct attaccatgg tgatgcggtt ttggcagtac 540
atcaatgggc gtggatagcg gtttgactca cggggatttc caagtctcca ccccattgac 600
gtcaatggga gtttgttttg gcaccaaaat caacgggact ttccaaaatg tcgtaacaac 660
tccgccccat tgacgcaaat gggcggtagg cgtgtacggt gggaggtcta tataagcaga 720
gctcgtttag tgaaccgtca gatcgcctgg agacgccatc cacgctgttt tgacctccat 780
agaagacacc gggaccgatc cagcctccgc ggccgggaac ggtgcattgg aacgcggatt 840
ccccgtgcca agagtgacgt aagtaccgcc tatagagtct ataggcccac cccttggct 900
tcttatgcat gctatactgt ttttggcttg 930

Reference: Cell 41:521-530, 1985

3. HCMV promoter, exon 1, intron A and part of exon 2; M60321
: (SEQ ID NO:50)
ctgcagtgaa taataaaatg tgtgtttgtc cgaaatacgc gttttgagat ttctgtcgcc 60
gactaaattc atgtcgcgcg atagtggtgt ttatcgccga tagagatggc gatattggaa 120
aaatcgatat ttgaaaatat ggcatattga aaatgtcgcc gatgtgagtt tctgtgtaac 180

tgatatcgcc atttttccaa aagtgatttt tgggcatacg cgatatctgg cgatacggct 240
tatatcgttt acgggggatg gcgatagacg actttggcga cttgggcgat tctgtgtgtc 300
gcaaatatcg cagtttcgat ataggtgaca gacgatatga ggctatatcg ccgatagagg 360
cgacatcaag ctggcacatg gccaatgcat atcgatctat acattgaatc aatattggca 420
attagccata ttagtcattg gttatatagc ataaatcaat attggctatt ggccattgca 480
tacgttgtat ctatatcata atatgtacat ttatattggc tcatgtccaa tatgaccgcc 540
atgttgacat tgattattga ctagttatta atagtaatca attacggggt cattagttca 600
tagcccatat atggagttcc gcgttacata acttacggta aatggcccgc ctcgtgaccg 660
cccaacgacc cccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata 720
gggactttcc attgacgtca atgggtggag tatttacggt aaactgccca cttggcagta 780
catcaagtgt atcatatgcc aagtccggcc ccctattgac gtcaatgacg gtaaatggcc 840
cgcctggcat tatgcccagt acatgacctt acgggacttt cctacttggc agtacatcta 900
cgtattagtc atcgctatta ccatggtgat gcggttttgg cagtacacca atgggcgtgg 960
atagcggttt gactcacggg gatttccaag tctccacccc attgacgtca atgggagttt 1020
gttttggcac caaaatcaac gggactttcc aaaatgtcgt aataaccccg ccccgttgac 1080
gcaaatgggc ggtaggcgtg tacggtggga ggtctatata agcagagctc gtttagtgaa 1140
ccgtcagatc gcctggagac gccatccacg ctgttttgac ctccatagaa gacaccggga 1200
ccgatccagc ctccgcggcc gggaacggtg cattggaacg cggattcccc gtgccaagag 1260
tgacgtaagt accgcctata gactctatag gcacacccct ttggctctta tgcatgctat 1320
actgtttttg gcttggggcc tatacacccc cgctccttat gctataggtg atggtatagc 1380
ttagcctata ggtgtgggtt attgaccatt attgaccact cccctattgg tgacgatact 1440
ttccattact aatccataac atggctcttt gccacaacta tctctattgg ctatatgcca 1500
atactctgtc cttcagagac tgacacggac tctgtatttt tacaggatgg ggtcccattt 1560
attatttaca aattcacata tacaacaacg ccgtcccccg tgcccgcagt ttttattaaa 1620
catagcgtgg gatctccacg cgaatctcgg gtacgtgttc cggacatggg ctcttctccg 1680
gtagcggcgg agcttccaca tccgagccct ggtcccatgc ctccagcggc tcatggtcgc 1740
tcggcagctc cttgctccta acagtggagg ccagacttag gcacagcaca atgcccacca 1800
ccaccagtgt gccgcacaag gccgtggcgg tagggtatgt gtctgaaaat gagctcggag 1860
attgggctcg caccgtgacg cagatggaag acttaaggca gcggcagaag aagatgcagg 1920
cagctgagtt gttgtattct gataagagtc agaggtaact cccgttgcgg tgctgttaac 1980
ggtggagggc agtgtagtct gagcagtact cgttgctgcc gcgcgcgcca ccagacataa 2040
tagctgacag actaacagac tgttcctttc catgggtctt ttctgcagtc accgtccttg 2100
acacgatgga gtcctctgcc aagagaaaga tggaccctga taatcctgac gagggccctt 2160
cctccaaggt gccacggtac gtgtcggggt ttgtgccccc ccttttttct ataaaattgt 2220
attaatgtta tatacatatc tcctgtatgt gacccatgtg cttatgactc tatttctcat 2280
gtgtttaggc ccgagacacc cgtgaccaag gccacgacgt tcctgcagac tatgttgagg 2340
aaggaggtta acagtcagct g 2361

Reference: Nucleic Acids Res. 19, 3979-3986 (1991)

4. HCMV promoter/enhancer with upstream NF1 binding sites; includes 1140bp of upstream promoter with 748bp of exon 1 and intron A; X03922
: (SEQ ID NO:51

ctgcagtgaa taataaaatg tgtgtttgtc cgaaatacgc gtttgagatt tctgtcccga 60
ctaaattcat gtcgcgcgat agtggtgttt atcgccgata gagatggcga tattggaaaa 120
atcgatattt gaaaatatgg catattgaaa atgtcgccga tgtgagtttc tgtgtaactg 180
atatcgccat ttttccaaaa gttgattttt gggcatacgc gatatctggc gatacgctta 240
tatcgtttac gggggatggc gatagacgcc tttggtgact tgggcgattc tgtgtgtcgc 300
aaatatcgca gtttcgatat aggtgacaga cgatatgagg ctatatcgcc gatagaggcg 360
acatcaagct ggcacatggc caatgcatat cgatctatac attgaatcaa tattggccat 420
tagccatatt attcattggt tatatagcat aaatcaatat tggctattgg ccattgcata 480
cgttgtatcc atatcataat atgtacattt atattggctc atgtccaaca ttaccgccat 540
gttgacattg attattgact agttattaat agtaatcaat tacggggtca ttagttcata 600
gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgcct ggctgaccgc 660
ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta acgccaatag 720
ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac ttggcagtac 780
atcaagtgta tcatatgcca agtacgcccc ctattgacgt caatgacggt aaatggcccg 840
cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag tacatctacg 900

tattagtcat cgctattacc atggtgatgc ggttttggca gtacatcaat gggcgtggat 960
agcggtttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat gggagtttgt 1020
tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caactccgcc ccattgacgc 1080
aaatgggcgg taggcgtgta cggtgggagg tctatataag cagagctcgt ttagtgaacc 1140
gtcagatcgc ctggagacgc catccacgct gttttgacct ccatagaaga caccgggacc 1200
gatccagcct ccgcggccgg gaacggtgca ttggaacgcg gattccccgt gccaagagtg 1260
acgtaagtac cgcctataga gtctataggc ccacccccct ggcttcttat gcatgctata 1320
ctgtttttgg cttggggtct atacacccca gcttcctcat gttataggtg atggtatagc 1380
ttagcctata ggtgtgggtt attgaccatt attgaccact cccctattgg tgacgatact 1440
ttccattact aatccataac atggctcttt gcacaactct ctttattggc tatatgccaa 1500
tacactgtcc ttcagagact gacacggact ctgtattttt acaggatggg gtctcattta 1560
ttatttacaa attcacatat acaacaccac cgtccccagt gcccgcagtt tttattaaac 1620
ataacgtggg atctccagcg aatctcgggt acgtgttccg gacatggggc tcttctccgg 1680
tagcggcgga gcttctacat ccagccctgc tcccatcctc ccactcatgg tcctcggcag 1740
ctccttgctc ctaacagtgg aggccagact taggcacagc acgatgccca ccaccaccag 1800
tgtgcccaca aggccgtggc ggtagggtat gtgtctgaaa atgagctc 1848

Reference: EMBO J. 5 (6), 1367-1371 (1986)

5. Various strains of HMCV IE promoter/enhancer; these are different from each other at a few residues compared to the two sequences listed above in 1 and 2; M64940-M64944

M64940
: (SEQ ID NO:52)

ggcacatggc caatgcatat cgatatatac attgaatcaa tattggctat tagccatatt 60
agtcattggt tatatagcat aaatcaatat tggctaatgg ccattgcata cattgcagct 120
atagcataat atgtacattt atattggctc atgtccaata tgaccgccat gttgacattg 180
attattgact agttattaat agtaatcaat tacggggtca ttagttcata gcccatatat 240
ggagttcccg cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc 300
cccgcccatt gacgtcaata atgacgtgag ttcccatagt aacgccaata gggactttcc 360
attgacgtca atgggaggag tatttacggt aaactgccca cttggcagta catcaagtgt 420
atcatatgcc aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt 480
atgcccagta catgaccttа cgggactttc ctacttggca gtacatctac gtattagtca 540
tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg 600
actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc 660
aaattcaacg ggactttcca aaatgtcgta ataactccgc cccattgacg caaatgggcg 720
gtaggcgtgt acgatgggtg gtctatataa gcagagctcg tttagtgaac cgtcagatcg 780
cctggagacg ccatccacgc tgttttgacc tccatagaag acaccgggac cgatccagcc 840
tccgcggccg ggaacggtgc attggaacgc ggattc 876

M64941
: (SEQ ID NO:53)

ggcacatggc caatgcatat cgatatatac attgaatcaa tattggccat tagccatatt 60
agtcattggt tatatagcat aaatcaatat tggctaatgg ccattgcata cgttgcatct 120
atatcataat gtgtacattt atattggctc atgtccaata tgaccgccat gttgacattg 180
attattgact agttattaat agtaatcaat tacggggtca ttagttcata gcccatatat 240
ggagttccgc gttacataac ttacggtaaa tggcccgcct ggctgaccgc ccaacgaccc 300
ccgcccattg acgtcaataa tgacgtgggt tcccatagta acgccaatag ggactttcca 360
ttgacgtcaa tgggaggagt atttacggta aactgcccac ttggcagtac atcaagtgta 420
tcatatgcca agtacgcccc ctattgacgt caatgacggt aaatggcccg cctggcatta 480
tgcccagtac atgaccttac gggactttcc tacttggcag tacatctacg tattagtcat 540
cgctattacc atggtgatgc ggttttggca gtacatcaat gggcgtggat agcggtttga 600
ctcacgggga tttccaagtc tccaccccat tgacgtcaat gggagtttgt tttggcacca 660
aattcaacgg gactttccaa aatgtcgtaa taactccgcc ccattgacgc aaatgggcgg 720
taggcgtgta ctatgggagg tctatataag cagagctcgt ttagtgaacc gtcagatcgc 780
ctggagacgc catccacgct gttttgacct ccatagaaga caccgggacc gatccagcct 840
ccgcggccgg gaacggtgca ttggaacgcg gattc 875

M64942
: (SEQ ID NO:54)

```
ggcacatggc caatgcatat cgatatatac attgaatcaa tattggctat tagccatatt 60
agtcattggt tatatagcat aaatcaatat tggctaatgg ccattgcata cattgcagct 120
atagcataat atgtacattt atattggctc atgtccaata tgaccgccat gttgacattg 180
attattgact agttattaat agtaatcaat tacggggtca ttagttcata gcccatatat 240
ggagttcccg cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc 300
cccgcccatt gacgtcaata ttgacgtgag ttcccatagt aacgccaata gggactttcc 360
attgacgtca atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt 420
atcatatgcc aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt 480
atgcccagta catgacctta cgggactttc ctacttggca gtacatctac gtattagtca 540
tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg 600
actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc 660
aaattcaacg ggactttcca aaatgtcgta ataactccgc cccattgacg caaatgggcg 720
gtaggcgtgt acgattggga ggtctatata agcagagctc gtttagtgaa ccgtcagatc 780
gcctggagac gccatccacg ctgttttgac ctccatagaa gacaccggga ccgatccagc 840
ctccgcggcc gggaacggtg cattggaacg cggattc                          877
```

M64943
: (SEQ ID NO:55)

```
ggcacatggc caatgcatat cgatctatac attgaatcaa tattggccat tagccatatt 60
agtcattggt tatatagcat aaatcaatat tgactattgg ccattgcata cgttgtatcc 120
atatcataat atgtacattt atattggctc atgtccaata tgaccgccat gttgacattg 180
attattgact agttattaat agtaatcaat tacagggtca ttagttcata gcccatatat 240
ggagttccgc gttacataac ttacggtaaa tggcccgcct ggctgaccgc ccaacgaccc 300
ccgcccattg acgtcaataa cgacgtatgt tcccatagta acgctaatag ggactttcca 360
ttgacgtcaa tgggaggagt atttacggta aactgcccac ttggcagtac atcaagtgta 420
tcatatgcca agtacgcccc ccattgacgt caatgacggt aaatggcccg cctggcatta 480
tgcccagtac atgaccttac gggactttcc tacttggcag tacatctacg tattagtcat 540
cactattacc atggtgatgc ggttttggca gtacatcaat gggtgtggat agcggtttga 600
ctcacgggga tttccaagtc tccaccccat tgacgtcaat gggagtttgt tttggcacca 660
aaatcaacgg gactttccaa aatgtcgtaa taactccgcc ccattgacgc aaatgggcgg 720
taggcgtgta cagtgggagg tctatataag cagagctcgt ttagtgaacc gtcagatcgc 780
ctggagacgc catccacgct gttttgacct ccatagaaga caccgggacc gatccagcct 840
ccgcggccgg gaacggtgca ttggaacgcg gatt                             874
```

M64944
: (SEQ ID NO:56)

```
ggcacatggc caatgcatat cgatatatac attgaatcaa tattggccat tagccatatt 60
agtcattggt tatatagcgt aaatcaatat tggctaatgg ccatcgcata cgttgcatct 120
atatcataat gtgtacattt atattggctc atgtccaata tgaccgccat gttgacattg 180
attattgact agttattaat agtaatcaat tacggggtca ttagttcata gcccatatat 240
ggagttcccg cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc 300
cccgcccatt gacgtcaata atgacgtgag ttcccatagt aacgccaata gggactttcc 360
attgacgtca atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt 420
atcatatgcc aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt 480
atgcccagta catgacctta cgggactttc ctacttggca gtacatctac gtattagtca 540
tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg 600
actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc 660
aaattcaacg ggactttcca aaatgtcgta ataactccgc cccattgacg caaatgggcg 720
gtaggcgtgt actatgggag gtctatataa gcagagctcg tttagtgaac cgtcagatcg 780
cctggagacg ccatccacgc tgttttgacc tccatagaag acaccgggac cgatccagcc 840
tccgcggccg ggaacggtgc attggaacgc ggattc                           876
```

Reference: J. Clin. Microbiol. 29, 2494-2502 (1991)

6. SV40 polyadenylation signal (late and early); J02400
: (SEQ ID NO:57)

ggggatccag acatgataag atacattgat gagtttggac aaaccacaac tagaatgcag 60
tgaaaaaaat gctttatttg tgaaatttgt gatgctattg ctttatttgt aaccattata 120
agctgcaata aacaagttaa caacaacaat tgcattcatt ttatgtttca ggttcagggg 180
gaggtgtggg aggtttttta aagcaagtaa aacctctaca aatgtggtat ggctgattat 240
gatcatgaac 250

Reference: Proc. Natl. Acad. Sci. U.S.A. 78 (1), 100-104 (1981)

7. Rabbit βglobin intron 2; J00600
: (SEQ ID NO:58)

ggatcctgag aacttcaggg tgagtttggg gacccttgat tgttctttct ttttcgctat 60
tgtaaaattc atgttatatg gaggggggcaa agttttcagg gtgttgttta gaatgggaag 120
atgtcccttg tatcaccatg gaccctcatg ataattttgt ttctttcact ttctactctg 180
ttgacaacca ttgtctcctc ttatttcttt ttcattttct gtaacttttt cgttaaactt 240
tagcttgcat ttgtaacgaa tttttaaatt cacttttgtt tatttgtcag attgtaagta 300
ctttctctaa tcacttttttt ttcaaggcaa tcagggtata ttatattgta cttcagcaca 360
gttttagaga acaattgtta taattaaatg ataaggtaga atatttctgc atataaattc 420
tggctggcgt ggaaatattc ttattggtag aaacaactac accctggtca tcatcctgcc 480
tttctcttta tggttacaat gatatacact gtttgagatg aggataaaat actctgagtc 540
caaaccgggc ccctctgcta accatgttca tgccttcttc tctttcctac agctcctggg 600
caacgtgctg 610

References: Cell 10, 549-558 (1977); Cell 18, 1285-1297 (1979)

8. Minimal synthetic rabbit βglobin polyadenylation signal
: (SEQ ID NO:59)

aataaaagat ccagagctct agagatctgt gtgttggttt tttgtgtg 48

Reference: Genes and Development 3: 1019-1025, 1989

V. IL-18 sequences to claim

1. Mature consensus human IL-18 linked to an HC signal sequence, with intron included and underlined. Bold areas are from the HC signal sequence, and the unbolded are the linked mature human IL-18 sequence
: (SEQ ID NO:60)

ATGGGGTCAACCGCCATCCTCGGCCTCCTCCTGGCTGTTCTCCAAGGTCAGTCCTGCCGAGGTCTTGAGG
TCACAGAGGAGAACGGGTGGAAAGGAGCCCCTGATTCAAATTTTGTGTCTCCCCCACAGGAGTCTGTGCC
tacttt ggcaagctt gaatctaaat tatcagtcat aagaaatttg aatgaccaag
ttctcttcat tgaccaagga aatcggcctc tatttgaaga tatgactgat tctgactgta
gagataatgc accccggacc atatttatta taagtatgta taaagatagc cagcctagag
gtatggctgt aactatctct gtgaagtgtg agaaaatttc aactctctcc tgtgagaaca
aaattatttc ctttaaggaa atgaatcctc ctgataacat caaggataca aaaagtgaca
tcatattctt tcagagaagt gtcccaggac atgataataa gatgcaattt gaatcttcat
catacgaagg atactttcta gcttgtgaaa aagagagaga ccttttttaaa ctcattttga
aaaaagagga tgaattgggg gatagatcta taatgttcac tgttcaaaac gaagactag

: (SEQ ID NO:61)

atggggtcaa ccgccatcct cggcctcctc ctggctgttc tccaaggtca gtcctgccga 60
ggtcttgagg tcacagagga gaacgggtgg aaaggagccc ctgattcaaa ttttgtgtct 120
cccccacagg agtctgtgcc tactttggca agcttgaatc taaattatca gtcataagaa 180
atttgaatga ccaagttctc ttcattgacc aaggaaatcg gcctctattt gaagatatga 240

ctgattctga ctgtagagat aatgcacccc ggaccatatt tattataagt atgtataaag 300
atagccagcc tagaggtatg gctgtaacta tctctgtgaa gtgtgagaaa atttcaactc 360
tctcctgtga gaacaaaatt atttccttta aggaaatgaa tcctcctgat aacatcaagg 420
atacaaaaag tgacatcata ttctttcaga gaagtgtccc aggacatgat aataagatgc 480
aatttgaatc ttcatcatac gaaggatact ttctagcttg tgaaaaagag agagaccttt 540
ttaaactcat tttgaaaaaa gaggatgaat tgggggatag atctataatg ttcactgttc 600
aaaacgaaga ctag 614

: (SEQ ID NO:62)
ATGGGGTCAACCGCCATCCTCGGCCTCCTCCTGGCTGTTCTCCAAGGTCAGTCCTGCC
GAGGTCTTGAGGTCACAGAGGAGAACGGGTGGAAAGGAGCCCCTGATTCAAATTTT
GTGTCTCCCCCACAGGAGTCTGTGCC

atggggtcaa ccgccatcct cggcctcctc ctggctgttc tccaaggtca gtcctgccga 60
ggtcttgagg tcacagagga gaacgggtgg aaaggagccc ctgattcaaa ttttgtgtct 120
cccccacagg agtctgtgcc 140

: (SEQ ID NO:63)
tacttt ggcaagctt gaatctaaat tatcagtcat aagaaatttg aatgaccaag ttctcttcat tgaccaagga aatcggcctc tatttgaaga tatgactgat tctgactgta gagataatgc accccggacc atatttatta taagtatgta taaagatagc cagcctagag gtatggctgt aactatctct gtgaagtgtg agaaaatttc aactctctcc tgtgagaaca aaattatttc ctttaaggaa atgaatcctc ctgataacat caaggataca aaaagtgaca tcatattctt tcagagaagt gtcccaggac atgataataa gatgcaattt gaatcttcat catacgaagg atactttcta gcttgtgaaa aagagagaga ccttttaaa ctcattttga aaaaagagga tgaattgggg gatagatcta taatgttcac tgttcaaaac gaagactag

tactttggca agcttgaatc taaattatca gtcataagaa atttgaatga ccaagttctc 60
ttcattgacc aaggaaatcg gcctctattt gaagatatga ctgattctga ctgtagagat 120
aatgcacccc ggaccatatt tattataagt atgtataaag atagccagcc tagaggtatg 180
gctgtaacta tctctgtgaa gtgtgagaaa atttcaactc tctcctgtga gaacaaaatt 240
atttccttta aggaaatgaa tcctcctgat aacatcaagg atacaaaaag tgacatcata 300
ttctttcaga gaagtgtccc aggacatgat aataagatgc aatttgaatc ttcatcatac 360
gaaggatact ttctagcttg tgaaaaagag agagaccttt ttaaactcat tttgaaaaaa 420
gaggatgaat tgggggatag atctataatg ttcactgttc aaaacgaaga ctag 474

: (SEQ ID NO:64)
MGSTAILGLLLAVLQGVCA

Met Gly Ser Thr Ala Ile Leu Gly Leu Leu Leu Ala Val Leu Gln Gly
1 5 10 15

Val Cys Ala

: (SEQ ID NO:65)
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEK
ISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKK
EDELGDRSIMFTVQNED

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1 5 10 15

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20 25 30

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile

```
        35                  40                  45

Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
    50                  55                  60

Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
65                  70                  75                  80

Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                85                  90                  95

Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
            100                 105                 110

Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
        115                 120                 125

Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
    130                 135                 140

Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145                 150                 155
```

2. Mature consensus human IL-18 linked to a human LC signal sequence, with no intron. Bold areas are from the LC signal sequence, and the unbolded are the linked mature human IL-18 sequence.

: (SEQ ID NO:66)

ATGGCCTGGACCGTTCTCCTCCTCGGCCTCCTCTCTCACTGCACAGGCTCTGTGACCTCC tacttt
ggcaagctt gaatctaaat tatcagtcat aagaaatttg aatgaccaag ttctcttcat
tgaccaagga aatcggcctc tatttgaaga tatgactgat tctgactgta gagataatgc
accccggacc atatttatta taagtatgta taaagatagc cagcctagag gtatggctgt
aactatctct gtgaagtgtg agaaaatttc aactctctcc tgtgagaaca aaattatttc
ctttaaggaa atgaatcctc ctgataacat caaggataca aaaagtgaca tcatattctt
tcagagaagt gtcccaggac atgataataa gatgcaattt gaatcttcat catacgaagg
atactttcta gcttgtgaaa aagagagaga ccttttaaa ctcattttga aaaaagagga
tgaattgggg gatagatcta taatgttcac tgttcaaaac gaagactag

```
atggcctgga ccgttctcct cctcggcctc ctctctcact gcacaggctc tgtgacctcc 60
tactttggca agcttgaatc taaattatca gtcataagaa atttgaatga ccaagttctc 120
ttcattgacc aaggaaatcg gcctctattt gaagatatga ctgattctga ctgtagagat 180
aatgcacccc ggaccatatt tattataagt atgtataaag atagccagcc tagaggtatg 240
gctgtaacta tctctgtgaa gtgtgagaaa atttcaactc tctcctgtga gaacaaaatt 300
atttccttta aggaaatgaa tcctcctgat aacatcaagg atacaaaaag tgacatcata 360
ttctttcaga gaagtgtccc aggacatgat aataagatgc aatttgaatc ttcatcatac 420
gaaggatact ttctagcttg tgaaaaagag agagaccttt ttaaactcat tttgaaaaaa 480
gaggatgaat tgggggatag atctataatg ttcactgttc aaaacgaaga ctag       534
```

: (SEQ ID NO:67)

ATGGCCTGGACCGTTCTCCTCCTCGGCCTCCTCTCTCACTGCACAGGCTCTGTGACCTCC

```
atggcctgga ccgttctcct cctcggcctc ctctctcact gcacaggctc tgtgacctcc 60
```

: (SEQ ID NO:68)

```
tacttt ggcaagctt gaatctaaat tatcagtcat aagaaatttg aatgaccaag
ttctcttcat tgaccaagga aatcggcctc tatttgaaga tatgactgat
tctgactgta gagataatgc accccggacc atatttatta taagtatgta
taaagatagc cagcctagag gtatggctgt aactatctct gtgaagtgtg
```

agaaaatttc aactctctcc tgtgagaaca aaattatttc ctttaaggaa
atgaatcctc ctgataacat caaggataca aaaagtgaca tcatattctt
tcagagaagt gtcccaggac atgataataa gatgcaattt gaatcttcat
catacgaagg atactttcta gcttgtgaaa aagagagaga ccttttaaa
ctcattttga aaaaagagga tgaattgggg gatagatcta taatgttcac
tgttcaaaac gaagactag

tactttggca agcttgaatc taaattatca gtcataagaa atttgaatga ccaagttctc 60
ttcattgacc aaggaaatcg gcctctattt gaagatatga ctgattctga ctgtagagat 120
aatgcacccc ggaccatatt tattataagt atgtataaag atagccagcc tagaggtatg 180
gctgtaacta tctctgtgaa gtgtgagaaa atttcaactc tctcctgtga gaacaaaatt 240
atttccttta aggaaatgaa tcctcctgat aacatcaagg atacaaaaag tgacatcata 300
ttctttcaga gaagtgtccc aggacatgat aataagatgc aatttgaatc ttcatcatac 360
gaaggatact ttctagcttg tgaaaaagag agagaccttt ttaaactcat tttgaaaaaa 420
gaggatgaat tgggggatag atctataatg ttcactgttc aaaacgaaga ctag 474

: (SEQ ID NO:69)
MAWTVLLLGLLSHCTGSVTSYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS
MYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSY
EGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED

Met Ala Trp Thr Val Leu Leu Leu Gly Leu Leu Ser His Cys Thr Gly
1 5 10 15

Ser Val Thr Ser Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile
20 25 30

Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro
35 40 45

Leu Phe Glu Asp Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg
50 55 60

Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met
65 70 75 80

Ala Val Thr Ile Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys
85 90 95

Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile
100 105 110

Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly
115 120 125

His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe
130 135 140

Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys
145 150 155 160

Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu
165 170 175

Asp

: (SEQ ID NO:70)
MAWTVLLLGLLSHCTGSVTS

```
Met Ala Trp Thr Val Leu Leu Leu Gly Leu Leu Ser His Cys Thr Gly
1               5                   10                  15

Ser Val Thr Ser
            20
```

: (SEQ ID NO:71)

```
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEK
ISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKK
EDELGDRSIMFTVQNED

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1               5                   10                  15

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
            20                  25                  30

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
        35                  40                  45

Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
    50                  55                  60

Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
65                  70                  75

Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                85                  90                  95

Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
            100                 105                 110

Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
        115                 120                 125

Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
    130                 135                 140

Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145                 150
```

Several changes could be made in IL-18, e.g., as presented herein. Changes in non-surface exposed residues that could be made that would result in the high probability of retention of IL-18 activity with no changes in immunogenicity are:

Thr^{10} for Ser^{10}
Val^{12} for Ile^{12}
Ser^{45} for Thr^{45}
Tyr^{47} for Phe^{47}
Phe^{52} for Tyr^{52}
Val^{64} for Ile^{64}
Tyr^{101} for Phe^{101}

These compounds would be useful as IL-18 agonists, for raising anti-IL-18 antibodies, for assays for IL-18 or IL-18 binding proteins and for preparation of affinity columns for the purification of IL-18 binding proteins.

Changes in amino acids with a low percentage of surface exposure that could be made that would result in the high probability of retention of IL-18 activity with possible changes in immunogenicity are:

Val^{5} for Leu^{5}
Val^{20} for Leu^{20}
Ile^{20} for Leu^{20}
Tyr^{21} for Phe^{21}
Val^{22} for Ile^{22}
Ile^{66} for Val^{66}
Thr^{72} for Ser^{72}
Phe^{148} for Ser^{148}

These compounds would be useful as IL-18 agonists, for raising anti-IL-18 antibodies, for assays for I-18 or IL-18 binding proteins and for preparation of affinity columns for the purification of IL-18 binding proteins.

Changes that could be made in amino acids involved in receptor contact that would result in alteration of IL-18 activity by either increasing or decreasing binding of the IL-18 analog to the IL-18 receptor are:

Glu^{4} for Lys^{4}
Ile^{6} for Glu^{6}
Asp^{8} for Lys^{8}
Ile^{13} for Arg^{13}
Arg^{15} for Leu^{15}
Lys^{17} for Asp^{17}
Lys^{27} for Arg^{27}
Ala^{30} for Phe^{30}
Lys^{35} for Asp^{35}
Phe^{37} for Asp^{37}
Glu^{38} for Cys^{38}
Ala^{39} for Arg^{39}
Trp^{40} for Asp^{40}
Glu^{51} for Met^{51}
Gly^{53} for Lys^{53}
Ile^{56} for Gln^{56}
Ala^{58} for Arg^{58}
Lys^{62} for Val^{62}
Lys^{94} for Asp^{94}
Phe^{95} for Thr^{95}
Leu^{104} for Arg^{104}
Ile^{108} for Gly^{108}

Lys^{111} for Asn^{111}
Phe^{129} for Lys^{129}
Asp^{131} for Arg^{131}
Leu^{132} for Asp^{132}
Glu^{133} for Leu^{133}
Ala^{134} for Phe^{134}
Thr^{150} for Met^{150}
Ser^{151} for Phe^{151}

Depending on the alteration of receptor binding or receptor activity, these compounds would be useful as IL-18 agonists or antagonists, for preparation of antibodies against IL-18, in assays for IL-18 or IL-18 binding proteins and the preparation of affinity columns for the purification of IL-18 binding proteins.

3. Other claimed changes in mature human IL-18 protein sequence:

a. Human sequence reference AF380360-1, linked to either signal sequence listed above, with the following sequence of mature human IL-18; this appears to be a natural variant of human IL-18, with changes in blue.

: (SEQ ID NO:72)

YFGKLESK LSVIRNLNNQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQPRGMAVTISV
KCEKISTLSCENKIISFKEVNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEGYF
LTCEKERDLFKLILKKEDELGDRSIMFTVQNED

```
Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1               5                  10                  15

Asn Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
            20                  25                  30

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
        35                  40                  45

Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
    50                  55                  60

Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
65                  70                  75                  80

Ile Ser Phe Lys Glu Val Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                85                  90                  95

Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
            100                 105                 110

Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Thr Cys Glu
        115                 120                 125

Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
    130                 135                 140

Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145                 150                 155
```

(SEQ ID NO:73)
tactttggca agcttgaatc taaattatca gtcataagaa atttgaataa ccaagttctc 60
ttcattgacc aaggaaatcg gcctctattt gaagatatga ctgattctga ctgtagagat 120
aatgcacccc ggaccatatt tattataagt atgtataaag atagccagcc tagaggtatg 180
gctgtaacta tctctgtgaa gtgtgagaaa atttcaactc tctcctgtga gaacaaaatt 240
atttccttta aggaagtgaa tcctcctgat aacatcaagg atacaaaaag tgacatcata 300
ttctttcaga gaagtgtccc aggacatgat aataagatgc aatttgaatc ttcatcatac 360
gaaggatact ttctaacttg tgaaaaagag agagaccttt ttaaactcat tttgaaaaaa 420
gaggatgaat tgggggatag atctataatg ttcactgttc aaaacgaaga ctag 474

b. Human sequence reference AAC27787; this appears to be a natural variant of human IL-18. Only mature human IL-18 protein is shown, DNA sequence is not available from database:

yfgklesklsvirnlndqvlfidqgnrplledmtdsdcrdnaprtifiirmykdsqprgmavtisvkcek
istlscenkiisfkemnppdnikdtksdiiffqrsvpghdnkmqfesssyegyflacekerdlfklilkk
edelgdrsimftvqsed

(SEQ ID NO:74)

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn
1				5					10					15	
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Leu	Glu	Asp
			20					25					30		
Met	Thr	Asp	Ser	Asp	Cys	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile
		35					40					45			
Ile	Arg	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile
	50					55					60				
Ser	Val	Lys	Cys	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile
65					70					75					80
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys
				85					90					95	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys
			100					105					110		
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu
		115					120					125			
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu
	130					135					140				
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Ser	Glu	Asp			
145					150					155					

c. Macaque sequence reference AF303732; mature macaque protein and DNA sequences are shown, and would be linked to either signal sequence shown above. Blue residues are altered from human consensus sequence:

(SEQ ID NO:75)

YFGKLESKLSIIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIINMYKDSQPRGMAVAISV
KCEKISTLSCENRIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKERDLYKL
ILKKKDELGDRSIMFTVQNED

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Ile Ile Arg Asn Leu Asn
1 5 10 15

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20 25 30

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35 40 45

Ile Asn Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Ala Ile
50 55 60

Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Arg Ile
65 70 75 80

Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
85 90 95

Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
100 105 110

Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
115 120 125

Lys Glu Arg Asp Leu Tyr Lys Leu Ile Leu Lys Lys Lys Asp Glu Leu
130 135 140

Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145 150 155

(SEQ ID NO:76)

tactttggca agcttgaatc taaattatca atcataagaa atttgaatga ccaagttctc 60
ttcattgacc aaggaaatcg gcccctattt gaagatatga ctgattctga ctgtagagat 120
aatgcacccc ggaccatatt tattataaat atgtataaag atagccagcc tagaggtatg 180
gctgtagcca tctctgtgaa atgtgagaaa atttcaactc tctcctgtga gaacagaatt 240
atttccttta aggaaatgaa tcctcctgat aacatcaagg atacgaaaag tgacatcata 300
ttctttcaga gaagtgtccc aggacatgat aataagatgc aatttgaatc ttcatcatac 360
gaaggatact ttctagcttg tgaaaaagag agagaccttt ataaactcat tttgaaaaaa 420
aaggatgaat tgggggatag atctataatg ttcactgttc aaaacgaaga ctag 474

Reference: J Interferon Cytokine Research 21:173-180, 2001, LD Giavedoni et al

d. Mutant human IL-18 with increased IL-18 activity and reduced ability to be inhibited by IL-18 binding protein; mature human IL-18 sequence with two altered residues indicated in blue:

(SEQ ID NO:77)

YFGKLASKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYADSQPRGMAVTISVKCEK
ISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKK
EDELGDRSIMFTVQNED

```
Tyr Phe Gly Lys Leu Ala Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1               5                  10                  15

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
            20                  25                  30

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
        35                  40                  45

Ile Ser Met Tyr Ala Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
    50                  55                  60

Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
65                  70                  75                  80

Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                85                  90                  95

Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
            100                 105                 110

Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
        115                 120                 125

Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
    130                 135                 140

Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145                 150                 155
```

Reference: PNAS 98:3304-3309, 2001 SM Kim et al.

Accordingly, based on the above non-limiting examples of specific substitutions, alternative substitutions can be made by routine experimentation, to provide alternative tumor/adjuvant vaccines of the present invention, e.g., by making one or more substitutions, insertions or deletions in proteins or tumor proteins which give rise to effective immune responses.

Amino acid sequence variations in a tumor protein or cytokine of the present invention can be prepared e.g., by mutations in the DNA. Such tumor or cytokine variants include, for example, deletions, insertions or substitutions of nucleotides coding for different amino acid residues within the amino acid sequence. Obviously, mutations that will be made in nucleic acid encoding a tumor protein or cytokine must not place the sequence out of reading frame and preferably will not create complementary domains that could produce secondary mRNA structures (see, e.g., Ausubel (1995

rev.), infra; Sambrook (1989), infra).

Tumor protein or cytokine-encoding nucleic acid of the present invention can also be prepared by amplification or site-directed mutagenesis of nucleotides in DNA or RNA encoding a tumor or cytokine protein or portion thereof, and thereafter synthesizing or reverse transcribing the encoding DNA to produce DNA or RNA encoding a tumor protein or cytokine variant (see, e.g., Ausubel (1995 rev.), infra; Sambrook (1989), infra), based on the teaching and guidance presented herein.

Recombinant viruses expressing tumor/adjuvant proteins of the present invention, or nucleic acid vectors encoding therefor, include a finite set of tumor/adjuvant-encoding sequences as substitution nucleotides that can be routinely obtained by one of ordinary skill in the art, without undue experimentation, based on the teachings and guidance presented herein. For a detailed description of protein chemistry and structure, see Schulz, G. E. et al., Principles of Protein Structure, Springer-Verlag, New York, N.Y. (1978), and Creighton, T. E., Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, Calif. (1983), which are hereby incorporated by reference. For a presentation of nucleotide sequence substitutions, such as codon preferences, see Ausubel et al., eds, Current Protocols in Molecular Biology, Greene Publishing Assoc., New York, N.Y. (1987-2001) (hereinafter, "Ausubel et al, sections A.1.1-A.1.24, and Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) at Appendices C and D.

Thus, one of ordinary skill in the art, given the teachings and guidance presented herein, will know how to substitute other amino acid residues in other positions of an DNA or RNA to obtain alternative tumor/adjuvant vaccines, including substitutional, deletional or insertional variants.

EXAMPLES

Screening Assays for Tumor Activity

For screening anti-tumor activity of sera or cells from an individual immunized with a vaccine of the invention, any known and/or suitable screening assay can be used, as is known in the art.

Specific Embodiment: Recombinant Vaccinia Virus Encoding tumor/adjuvant's, Nucleic acid vaccines and Methods of Making and Using Thereof

Overview. A suitable recombinant viral vector is used according to the present invention for expressing tumor proteins (e.g., MUC-1, PSA, KLK3 or any portion, variant or combination thereof) to provide at least a portion of a vaccine useful for the production, testing or use of a tumor vaccine of the present invention that induces at least one of a humoral or cellular immune response against the tumor, a portion thereof or a cell thereof, as well as for analyses of B-cell and CTL determinants.

A tumor vaccine of the present invention expresses at least one tumor nucleic acid or protein (tumor/adjuvant) and at least one adjuvant nucleic acid or protein. The tumor vaccine functionally encodes at least one tumor/adjuvant or adjuvant. Multiple, distinct fragments or plasmids encoding tumor/adjuvant and/or adjuvant (e.g., IL-18) can be prepared by substituting one tumor/adjuvant encoding sequence with another, e.g., using a restriction fragment or mutagenesis, according to known methods (see, e.g., Ausubel or Sambrook, supra).

Preparation of Tumor Vaccine. Methods for the preparation of individual plasmids (each expressing at least one unique tumor or adjuvant protein sequence) can utilize DNA or RNA amplification for the substitution of isolated protein variant sequences into a vector , which vector encodes a known tumor and/or adjuvant protein sequence, as known in the art.

Methods of amplification of RNA or DNA are well known in the art and can be used according to the present invention without undue experimentation, based on the teaching and guidance presented herein. Known methods of DNA or RNA amplification include, but are not limited to polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. Pat. Nos. 4,683,195, 4,683,202,

4,800,159, 4,965,188, to Mullis et al.; U.S. Pat. Nos. 4,795,699 and 4,921,794 to Tabor et al; U.S. Pat. No. 5,142,033 to Innis; U.S. Pat. No. 5,122,464 to Wilson et al.; U.S. Pat. No. 5,091,310 to Innis; U.S. Pat. No. 5,066,584 to Gyllensten et al; U.S. Pat. No. 4,889,818 to Gelfand et al; U.S. Pat. No. 4,994,370 to Silver et al; U.S. Pat. No. 4,766,067 to Biswas; U.S. Pat. No. 4,656,134 to Ringold) and RNA mediated amplification which uses anti-sense RNA to the target sequence as a template for double stranded DNA synthesis (U.S. Pat. No. 5,130,238 to Malek et al, with the trade name NASBA), the entire contents of which patents are herein entirely incorporated by reference.

For example, recombinant tumor vaccine constructs prepared by this route can be used for immunizations and elicitation of tumor-specific T and/or B-cell responses. Primers utilize conserved tumor sequences and thus successfully amplify genes from many diverse tumor patient or cell samples or from tumor nucleic acid libraries, as non-limiting examples. The basic techniques described here can similarly be used with PCR or other types of amplification primers, in order to substitute smaller or larger pieces of the sequence from field isolates for that found in vectors encoding a tumor protein. See, e.g., Ausubel; supra, Sambrook, supra.

Tumor/Adjuvant Encoding Nucleic Acids. The technique can use, as a non-limiting example, the isolation of DNA from tumor infected cells and the amplification of sequences by PCR. PCR or other amplification products provide the simplest means for the isolation of tumor sequences, but any other suitable and known methods can be used such as cloning and isolation of tumor/adjuvant encoding nucleic acid or proteins (see Ausubel, infra; Sambrook, infra). Enzyme restriction sites are preferably incorporated into PCR or other amplification primer sequences to facilitate gene cloning.

Isolated DNA for PCR can be prepared from multiple tumor or adjuvant sources, inclusive of fresh or frozen whole blood or tumor tissue or cells from tumor+ patients and cells that have been infected in vitro with tumor virus isolates.

In order to produce new tumor/adjuvant constructs, the polymerase chain reaction (PCR) is preferably used to amplify 100-2700 base pairs (bp) of a tumor protein encoding nucleic acid from each different tumor patient, tissue or cell sample. The PCR primers can represent well-conserved tumor sequences which are suitable for amplifying genes from known samples of genes, isolated tumors or diverse tumor patient samples. The amplified DNA preferably comprises a portion encoding 10-900 (such as 100-400, 400-600 or 600-900, or any range or value therein) amino acids of a PSA, MUC-1 or KLK-3 protein. Preferably, most or all of the entire gene is amplified. Optionally, the MUC-1 encoding sequence amplified is missing part or all of sequences encoding the 20 amino acid repeat or any combination or number of copies thereof, such but not limited, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 copies or any fraction thereof, such .1, .2, .3, .4, .5, .6, .7, .8, .9 of the encoding nucleic acid repeat, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 amino acids or any combination thereof. Non-limiting examples include 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, and the like, including any fractional amount thereof, such as .1, .2, and the like.

The PCR primers can be designed so that restriction enzyme sites flank the tumor protein or cytokine adjuvant gene sequence in a suitable expression plasmid or vector, such that they are incorporated into the amplified DNA products. Suitable host cells can then be transformed with the tumor/adjuvant plasmid(s) via any of a number of methods well-known in the art, including, e.g., electroporation, and recombinant colonies are picked and examined by sequencing.

Methods for the production of expression vectors are well-known in the art (see, e.g., Mackett, M. et al., Proc. Natl. Acad. Sci. (USA) 79:7415-7419 (1982); Panicali, D., and Paoletti, E., Proc. Natl. Acad. Sci. (USA) 79:4927-4931 (1982); U.S. Pat. No. 4,169,763; Mazzara, G. P. et al., Methods in Enz. 217:557-581 (1993), Ausubel et al., infra, (e.g., 16.15-16.19), each of which are entirely incorporated herein by reference).

For use in the present invention a nucleic acid vaccine or a viral vector vaccine can be either used alone, in combination or sequentially.

As a non-limiting example of a suitable viral vector for a tumor vaccine of the present invention, vaccinia virus has a number of useful characteristics, including capacity that permits cloning large fragments of foreign DNA (greater than 20 Kb), retention of infectivity after insertion of foreign DNA, a wide host range, a relatively high level of protein synthesis, and suitable transport, secretion, processing and post-translational modifications as dictated by the primary structure of the expressed protein and the host cell type use. For example, N-O-glycosylation, phosphorylation, myristylation, and cleavage, as well as assembly of expressed proteins, occur in a faithful manner.

Several variations of the vaccinia vector have been developed and are suitable for use in the present invention (e.g., see Ausubel et al., infra, sec. 16.15-16.19). Most commonly, after obtaining the virus stock (Ausubel, infra at sec. 16.16), a nucleic acid sequence encoding a tumor/adjuvant is placed under control of a vaccinia virus promoter and integrated into the genome of vaccinia so as to retain infectivity (Ausubel et al., infra at sec. 16.17). Alternatively, expression can be achieved by transfecting a plasmid containing the vaccinia promoter-controlled gene encoding a tumor/adjuvant into a cell that has been infected with wild-type vaccinia.

Preferably, the host cell and vector are suitable and approved for use in vaccination of mammals and humans. These recombinant vectors are then characterized using various known methods (Ausubel et al., infra at sec. 16.18). In still another variation, the bacteria phage T7 RNA polymerase chain can be integrated into the genome of the vector so that the tumor/adjuvant encoding sequences will be expressed under the control of a T7 promoter, either in transfected plasma, plasmid or a recombinant vaccinia virus, will be expressed.

The use of pox virus promoters is preferred for vaccinia expression because cellular and other viral promoters are not usually recognized by the vaccinia transcriptional apparatus. A compound early/late promoter is preferably used in recombinant vaccinia for nucleic acid vaccines, as it is desirable to express the tumor/adjuvant as an antigen

that is presented in recombinant vaccinia virus infected host cell in association with major histocompatibility class (MHC) I or II. Such MHC associated tumor protein will then form cytotoxic T cell targets, and prime vaccinated mammals for a cytotoxic T cell response and/or a humoral response against the expressed tumor tumor/adjuvants. This is because the ability of vaccinia viral vectors to induce MHC presentation in host cells for this type of antigen appears to diminish late in the infection stage. Transcripts originating early will terminate after the sequence TTTTTNT and lead to inadequate MHC presentation.

Alternatively, any such termination motifs within the coding sequence of the gene can be altered by mutagenesis if an early pox virus promoter is used, in order to enhance MHC presentation of protein antigens in host cells (Earl et al., infra, 1990). To mimic vaccinia virus mRNAs, untranslated leader and 3'-terminal sequences are usually kept short, if they are used in the vaccinia plasmids incorporating tumor/adjuvant encoding sequences.

Preferably, the plasmid used for making vaccinia constructs according to the present invention has been designed with restriction endonuclease sites for insertion of the gene downstream of the vaccinia promoter (Ausubel et al., infra, sec. 16.17). More preferably, the plasmid already contains an protein encoding sequence, wherein the restriction sites occur uniquely near each of the beginning and ends of the protein coding sequence. The same restriction fragment of the tumor/adjuvant encoding sequence can then replace the corresponding sequence in the plasmid. In such cases, the major portion of the tumor/adjuvant encoding sequence can be inserted after removing most or all of the protein encoding sequence from the plasmid.

Preferably, the resulting vaccinia construct (containing the tumor/adjuvant encoding sequence and the vaccinia promoter) is flanked by vaccinia DNA to permit homologous recombination when the plasmid is transfected into cells that have been previously infected with wild-type vaccinia virus. The flanking vaccinia virus DNA is chosen so that the recombination will not interrupt an essential viral gene.

Without selection, the ratio of recombinant to parental vaccinia virus is usually about

1:1000. Although this frequency is high enough to permit the use of plaque hybridization (see Ausubel et al., infra at sec. 6.3 and 6.4) or immunoscreening (Ausubel et al., infra at sec. 6.7) to pick recombinant viruses, a variety of methods to facilitate recombinant-virus identification have been employed. Nonlimiting examples of such selection or screening techniques are known in the art (see Ausubel et al., infra at sec. 16.17). Usually, the expression cassette is flanked by segments of the vaccinia thymidine kinase (TK) genes so that recombination results in inactivation of TK. Virus with a TK.sup.- phenotype can then be distinguished from those with a TK.sup.+ phenotype by infecting a TK.sup.- cell line in the presence of 5-bromo-deoxyuridine (5-BrdU), which must be phosphorylated by TK to be lethally incorporated into the virus genome. Alternatively or additionally, recombinant viruses can be selected by the co-expression of a bacterial antibiotic resistant gene such as ampicillin (amp) or guanine phosphoribosyl transferase (gpt). As a further example, co-expression of the Escherichia coli lac Z gene allows co-screening of recombinant virus plaques with Xgal (Ausubel, infra, sec. 16.17).

The recombinant vaccinia viruses expressing a tumor/adjuvant of the present invention can be optionally attenuated or inactivated according to known methods, such as by heat, paraformaldehyde treatment, ultraviolet irradiation, propriolactene treatment, hybrid or chimera formation or by other known methods (see, e.g., Zagury et al., Nature 332:728-731 (1988); Ito et al., Cancer Res. 50:6915-6918 (1990); Wellis et al., J. Immunol. 99:1134-9 (1967); D'Honcht, Vaccine 10 (Suppl.):548-52 (1992); Selenka et al., Arch. Hyg. Bakteriol. 153:244-253 (1969); Grundwald-Bearch et al., J. Cancer Res. Clin. Oncol. 117:561-567 (1991); the contents of which are entirely incorporated here by reference). For example, heat inactivation at 60.degree. C. will reduce virus titer considerably. Such attenuation techniques are safety tested, as incomplete inactivation might result in patient death (Dorozynski and Anderson, Science 252:501-502 (1991)).

Such attenuated or inactivated recombinant vaccinia is to be used where the patient may have a compromised immune system as complications or death can occur when live vaccinia is administered.

Pharmaceutical Compositions

Pharmaceutical preparations of the present invention, suitable for inoculation or for parenteral or oral administration, include a polyrecombinant virus vaccine comprising of at least 4, and up to about 10,000, preferably 4 to about 1000, and more preferably about 10 to about 100 different recombinant viruses, in the form of a cell lysate, membrane-bound fraction, partially purified, or purified form. Preferably, the nucleic acid vaccine comprises recombinant virus containing cell lysate (or membrane-bound fractions thereof) that further comprise tumor/adjuvant proteins already expressed by the recombinant viruses. The inclusion of the expressed tumor/adjuvants is now discovered to enhance the primary antibody response.

The nucleic acid vaccine composition can be in the form of sterile aqueous or non-aqueous solutions, suspensions, or emulsions, and can also contain auxiliary agents or excipients which are known in the art. Each of the at least about 4-20 different viruses encode and express a different tumor/adjuvant, as presented herein. tumor/adjuvants encoding DNA can be selected to represent tumor/adjuvants suitable for treatment. For example, a vaccine could represent sequences from any or any combination of suitable tumors and adjuvant proteins.

A nucleic acid vaccine composition can further comprise immunomodulators such as cytokines which accentuate an immune response to a viral infection. See, e.g., Berkow et al., eds., The Merck Manual, Fifteenth Edition, Merck and Co., Rahway, N.J. (1987); Goodman et al., eds., Goodman and Gilman's The Pharmacological Basis of Therapeutics, Eighth Edition, Pergamon Press, Inc., Elmsford, N.Y. (1990); Avery's Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics, Third Edition, ADIS Press, LTD., Williams and Wilkins, Baltimore, Md. (1987); and Katzung, ed. Basic and Clinical Pharmacology, Fifth Edition, Appleton and Lange, Norwalk, Conn. (1992), which references and references cited therein, are entirely incorporated herein by reference as they show the state of the art.

As would be understood by one of ordinary skill in the art, when a nucleic acid vaccine of the present invention is provided to an individual, it can be in a composition which

can further comprise at least one of salts, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the composition. Adjuvants are substances that can be used to specifically augment at least one immune response. Normally, the adjuvant and the composition are mixed prior to presentation to the immune system, or presented separately, but into the same site of the being immunized. Adjuvants can be loosely divided into several groups based upon their composition. These groups include oil adjuvants, mineral salts (for example, AlK(SO.sub.4).sub.2, AlNa(SO.sub.4).sub.2, AlNH.sub.4 (SO.sub.4), silica, kaolin, and carbon), polynucleotides (for example, poly IC and poly AU nucleic acids), and certain natural substances (for example, wax D from Mycobacterium tuberculosis, substances found in Corynebacterium parvum, or Bordetella pertussis, and members of the genus Brucella). Among those substances particularly useful as adjuvants are the saponins (e.g., Quil A., Superfos A/S, Denmark). Examples of materials suitable for use in vaccine compositions are disclosed, e.g., in Osol, A., ed., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. (1980), pp. 1324-1341, which reference is entirely incorporated herein by reference.

A pharmaceutical vaccine composition of the present invention can further or additionally comprise at least one antiviral chemotherapeutic compound. Non-limiting examples can be selected from at least one of the group consisting of gamma globulin, amantadine, guanidine, hydroxy benzimidazole, interferon-.alpha., interferon-.beta., interferon-.gamma., interleukin-16 (IL-16; Kurth, Nature, Dec. 8, 1995); thiosemicarbarzones, methisazone, rifampin, ribvirin, a pyrimidine analog (e.g., AZT and/or 3TC), a purine analog, foscarnet, phosphonoacetic acid, acyclovir, dideoxynucleosides, a protease inhibitor (e.g., saquinavir (Hoffmann-La Roche); indinavir (Merck); ritonavir (Abbott Labs); AG 1343 (Agouron Pharmaceuticals); VX-2/78 (Glaxo Wellcome)); chemokines, such as RANTES, MIP1.alpha. or MIP1.beta. (Science 270:1560-1561 (1995)) or ganciclovir. See, e.g., Richman: AIDs Res. Hum. Retroviruses 8: 1065-1071 (1992); Annu Rev Pharmacol Toxico 33: 149-164 (1993); Antimicrob Agents Chemother 37: 1207-1213 (1993); AIDs Res. Hum. Retroviruses 10: 901 (1994): Katzung (1992), infra, and the references cited therein on pages 798-800 and 680-681, respectively, which references are herein entirely incorporated by reference.

Pharmaceutical Uses

The administration of a vaccine (or the antisera which it elicits) can be for either a "prophylactic" or "therapeutic" purpose, and preferably for prophylactic purposes. When provided prophylactically, the nucleic acid vaccine composition is provided in advance of any detection or symptom of tumor associated pathology. The prophylactic administration of the compound(s) serves to prevent or attenuate any subsequent tumor associated pathology.

When provided therapeutically, the nucleic acid or viral vaccine is provided upon the detection of a symptom of actual infection. The administration of a vaccine after detection of tumor-associated pathology is provided only where the patient's immune system is determined to be capable of responding to administration of a vaccine of the present invention.

Alternatively, where the patient's immune response is compromised, therapeutic administration preferentially involves the use of an attenuated or inactivated viral vaccine composition where the viral vaccines are attenuated or inactivated, as presented above. See, e.g., Berkow (1987), infra, Goodman (1990), infra, Avery (1987), infra and Katzung (1992), infra, Dorozynski and Anderson, Science 252:501-502 (1991) which are entirely incorporated herein by reference, including all references cited therein.

A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. Such an agent is said to be administered in a "therapeutically or prophylactically effective amount" if the amount administered is physiologically significant. A vaccine or composition of the present invention is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient, preferably by enhancing a humoral or cellular immune response to a tumor.

The "protection" provided need not be absolute, i.e., the tumor need not be totally prevented or eradicated, provided that there is a statistically significant. improvement

relative to a control population. Protection can be limited to mitigating the severity or rapidity of onset of symptoms of the disease.

Pharmaceutical Administration

A vaccine of the present invention can confer resistance to one or more types of a tumor. The present invention thus concerns and provides a means for preventing or attenuating infection by at least one tumor. As used herein, a vaccine is said to prevent or attenuate a disease if its administration to an individual results either in the total or partial attenuation (i.e. suppression) of a symptom or condition of the disease, or in the total or partial immunity of the individual to the disease.

At least one nucleic acid vaccine of the present invention can be administered by any means that achieve the intended purpose, using a pharmaceutical composition as described herein.

For example, administration of such a composition can be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, transdermal, or buccal routes. Subcutaneous administration is preferred. Parenteral administration can be by bolus injection or by gradual perfusion over time. See, e.g., Berkow (1987), infra, Goodman (1990), infra, Avery (1987), infra, and Katzung (1992), infra, which are entirely incorporated herein by reference, including all references cited therein.

A typical regimen for preventing, suppressing, or treating a disease or condition which can be alleviated by a cellular immune response by active specific cellular immunotherapy, comprises administration of an effective amount of a vaccine composition as described is above, administered as a single treatment, or repeated as enhancing or booster dosages, over a period up to and including one week to about 24 months.

According to the present invention, an "effective amount" of a vaccine composition is one which is sufficient to achieve a desired biological effect, in this case at least one of

cellular or humoral immune response to at least one tumor. It is understood that the effective dosage will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The ranges of effective doses provided below are not intended to limit the invention and represent preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of skill in the art, without undue experimentation. See, e.g., Berkow (1987), infra, Goodman (1990), infra, Avery (1987), infra, Ebadi, Pharmacology, Little, Brown and Co., Boston, Mass. (1985), and Katsung (1992), infra, which references and references cited therein, are entirely incorporated herein by reference. Whatever dosage is used, it should be a safe and effective amount as determined by known methods, as also described herein.

Subjects

The recipients of the vaccines of the present invention can be any mammal which can acquire specific immunity via a cellular or humoral immune response to tumor, where the cellular response is mediated by an MHC class I or class II protein. Among mammals, the preferred recipients are mammals of the Orders Primata (including humans, chimpanzees, apes and monkeys). The most preferred recipients are humans.

Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration, and are not intended to be limiting of the present invention.

Examples

We believe it is preferable that cytotoxic immunity to MUC1 be generated through the expression of MUC1 by antigen presenting cells with the subsequent presentation of digested MUC1 peptides in the context of Class I molecules. Transgene has taken an approach along these lines, using a vaccinia virus encoding MUC1 and IL-2 (29-31). This strategy would allow expression of MUC1 with natural processing of peptide for presentation to the immune system, with the function of IL-2 being to support

the growth of CTLs. In three of nine patients, cellular responses were detected, and the two patients with documented CTL activity survived the longest, although the results are not significant (31). One important limitation to this strategy is that repeated administration of a viral vector results in a strong immune response to the vector itself. This limits the number of times the drug can be administered, because the host immune response acts to clear the drug very quickly. Another approach that may make its way to the clinic, and appears effective in mice, is the fusion of MUC1+ tumor cells with dendritic cells, followed by vaccination of the mice with the fusion cells (32, 33). This leads to specific MUC1 cellular immunity that is protective for tumor challenge and tumor treatment in mice. Because every patient is immunologically unique, this would require unique reagents for each patient. This approach may thus turn out to be very difficult to translate into mass usage because of its expense and requirement for sophisticated medical expertise.

Our strategy is to use DNA vaccination to drive a cellular immune response against tumor cells expressing MUC1. We believe that this approach offers significant advantages over the other strategies listed above. First, DNA vaccines are known to generate strong humoral and cellular immune responses in numerous animal studies (34, 35), and cellular responses in at least one human trial (36). Second, we believe that a cellular immune response, with the generation of CTLs will be the best way to eliminate MUC1+ tumor cells. CTLs directed against a particular antigen recognize specific peptides presented in the context of Class I molecules on a cell surface. Recognition by CTL then results in destruction of the cell expressing that antigen. DNA vaccines can induce the generation of CTLs directed against the antigen encoded by the vaccine (34, 35). If the antigen is a tumor antigen, tumor cells would be lysed by the CTLs. In contrast, anti-tumor antibodies are typically of low avidity and are not very effective in causing ADCC of tumor cells. Third, by injecting a plasmid that will encode the whole MUC1 protein, the patient's immune system can choose the best peptides for presentation according to his/her unique array of Class I molecules, rather than limiting the drug to one or several putative Class I peptides. Fourth, we have shown in preclinical studies that a combination of plasmids encoding MUC1 and the cytokine IL-18 protect mice from developing tumors, whereas plasmids encoding MUC1 or IL-18 alone offer little to no protection. IL-18 is a cytokine known to skew a nascent immune response toward a cellular response, rather than a humoral response (37). Fifth, DNA vaccination is a

flexible therapeutic strategy, in that one can design a DNA vaccine that encodes not just MUC1 but other molecules that could help to drive the immune response. Sixth, DNA vaccines are simple in concept and delivery to the patient, and should provide a cost-effective approach toward cancer treatment. Seventh, DNA vaccines can be administered indefinitely to the patient, because DNA is nontoxic, and because only the protein product of the DNA, not the DNA itself, is immunogenic.

The invention is a plasmid that encodes human MUC1 and a plasmid that encodes human IL-18, or a multicistron plasmid that encodes both genes. The mode of delivery could also be MUC1 DNA and IL-18 DNA encoded by a viral vector, or RNA encoding each gene. The invention includes an IL-18 gene construct comprised of mature IL-18 linked to a heterologous signal sequence, specifically an immunoglobulin signal sequence. This permits mature IL-18 to be expressed without the requirement for caspase cleavage of the IL-18 precursor protein.

Coinjection of both MUC1 and IL-18 plasmids intramuscularly at the same site is presumed to cause the local expression of both proteins in muscle cells, as well as the takeup and expression of both plasmids by professional antigen presenting cells (APCs) that are migrating through the tissue. This leads to a memory immune response that is protective for animals subsequently challenged with $MUC1^+$ tumor cells. It appears that the vaccination can break self-tolerance to MUC1.

The vaccination also leads to protection from subsequent challenge by $MUC1^-$ tumor cells that are otherwise identical to the $MUC1^+$ tumor cells. This phenomenon is known as epitope spreading, and may be a critical, unique feature of the vaccine that enables the immune system to develop a response to MUC1 and to other undefined antigens expressed by the tumor. Tumors are adept at evading the immune system, notably by changing their array of antigens on the cell surface (escape variants). Thus, a vaccine that induces immunity to more than one tumor antigen should make it more difficult for tumors to evade the immune system, and this could result in more effective cancer therapy.

Our studies show that MUC1 and IL-18 plasmids synergize to induce the formation of a protective anti-tumor immune response. The first study was performed in C57Bl/6 mice (43). Nine groups of animals were vaccinated with either vehicle control, empty vector, pMUC1, or pIL-18, singly or in combination. Three vaccinations were performed over a three-week period, and the mice were challenged with syngeneic

MUC1$^+$ tumor cells (38, 39) by subcutaneous injection in the fourth week. Animals were then monitored for tumor incidence and tumor volume for up to seven weeks thereafter. Results are shown in Figure 1. None of the mice in the groups receiving vehicle, empty plasmid or pIL-18 were protected from developing tumors. Two groups received suboptimal doses of pMUC1, and only 2-3 mice were protected. Of the groups vaccinated with the various combinations of pMUC1 and pIL-18 plasmids, those groups receiving the higher dose of pMUC1 in combination with either dose of pIL-18 showed good protection (6/9 or 7/9 mice). These results are significantly different from the control results ($p=0.011$ or $p=0.003$).

Tumor volume was also evaluated. The best result was seen in the group receiving 5ug pMUC1/5ug pIL-18, where tumor growth appeared to be delayed to day 35. At that time the slope of tumor growth parallels that of the other groups (Figure 2).

Sera from the animals was collected pre-study, and at days 13, 26 and 34 during and after vaccination. Sera were tested for the presence of anti-MUC1 antibodies, but only low titers were seen. This result indicates that a strong anti-MUC1 antibody response was not responsible for the protection seen in the animals.

The surviving mice from the first phase of this study were then entered into a second phase, which was designed to learn if the mice had developed a protective anti-tumor immune response that could be recalled. The mice were subjected to a second challenge with MUC1$^+$ tumor cells, with the results shown in Figure 3. Again, the group that originally received 5ug of each test plasmid fared well, with 4 of the original 9 mice protected for another 49 days, while in the group receiving 5ug pMUC1 and 50ug pIL-18, 3 of the original 9 mice were still protected. This result indicates that some of the rechallenged mice had developed a protective cellular immune response, because they were able to fend off a second challenge of tumor cells.

The above study showed that while neither plasmid alone offered much protection from tumor challenge, and thus did not prime the immune response particularly well, vaccination with both plasmids at certain doses could indeed lead to protection from tumor challenge, or at least a delay in tumor development. We then sought to reproduce these results in a model system more reflective of the human patient, and we used a strain of C57Bl/6 mice transgenic for human MUC1 (40-42; referred to as MUC1 Tg mice). This model would allow us to test if the combination of plasmids was effective, and if we could break tolerance to a self-antigen. We repeated the study shown above using the

transgenic mice and using increased doses of pMUC1, but testing the same doses of pIL-18.

The results in the second study are consistent with the first (44; see Figure 4). Animals receiving empty plasmid showed no protection from tumor challenge. Only one animal receiving the higher dose of pMUC1 was protected, while none of those receiving pIL-18 alone were protected. In contrast, the groups receiving the combinations of pMUC1/pIL-18 showed notable protection, particularly the group receiving the highest dose of each plasmid (8/9 without tumors; p=0.002).

On day 28 the tumors were excised and weighed, as shown in Figure 5. Neither the pMUC1 nor pIL-18 groups had mean weights that were significantly different from the empty vector control group. However, all four pMUC1/pIL-18 combination groups had mea tumor weights that were significantly smaller than those of the empty vector control group (p=0.004-0.038). The results show that not only did the combination of pMUC1/pIL-18 have a positive effect on tumor incidence, it had a positive effect on tumor weights as well. Neither of these effects was observed with either plasmid alone.

Mice from the combination groups were then rechallenged with $MUC1^+$ tumor cells to learn if they had developed protective immunity that could be recalled (Figure 6). Of the 5 mice that had originally been vaccinated with 100ug pMUC1/50ug pIL-18, 4/5 remained free of tumor growths in phase II after the second tumor challenge. Both of the mice from the group that was vaccinated with 100ug pMUC1/5ug pIL-18 also remained free of growths throughout the second challenge, while 1 of 2 mice each from the two remaining groups developed growths. The results support the hypothesis that the mice developed a memory response that was recalled in response to the second tumor challenge.

We then determined if the mice had developed a broader immune response to antigens besides MUC1. The same animals in phase II were challenged again but with $MUC1^-$ MC38 tumor cells. The MC38 cells are the parent line to the $MUC1^+$ tumor cells, and are otherwise expected to be identical (38). Results of the third challenge are shown in Figure 7. Interestingly, the mice that were originally vaccinated with the 100ug dose of pMUC1 in combination with either dose of pIL-18 continue to be protected, while the three naïve control MUC1 Tg mice succumbed to tumors. This result suggests that the vaccinated mice have developed immunity to determinants shared between the two cell lines, in addition to immunity to MUC1. This phenomenon is known as epitope

spreading, and is well documented in autoimmune disease models in animals (46, 47). In these models, animals are first immunized with a self-protein or peptide against which they develop immunity, and the immune response causes the destruction of normal tissue expressing the native protein. After tissue destruction, the immune response broadens to include antigens that the animals were not immunized against but which are expressed by the target tissue. If such a process could be duplicated in humans, DNA vaccination could be very effective at inducing immunity to MUC1 as well as other unique determinants present on tumor cells, and broadening the immune response should only be helpful to patient therapy. In addition, tumor cells are continuously changing in response to environmental pressures, and therapy against one antigen could lead to remission until escape variants arise that no longer express that antigen. With epitope spreading, the immune response broadens to include other antigens and theoretically should improve the chances that the tumor cells will be unable to escape the vigilance of the immune system.

A second advantage of this approach includes the use of a human IL-18 construct that encodes the mature form of IL-18 linked to an immunoglobulin signal sequence. IL-18 is ordinarily expressed as a precursor protein that is not functional until it is cleaved into its mature form by caspase (48, 49). Most cells do not express caspase, therefore one strategy to ensure IL-18 expression in any cell type is to engineer the protein so that it does not require caspase cleavage for maturation. We have used a genomic fragment that encodes the anti-IL-12 12B75 heavy chain signal sequence (50) linked to a human IL-18 cDNA sequence to ensure production of human IL-18 in any cell type. This strategy was effective for both the human and mouse IL-18 genes.

A third advantage of our approach is to use a MUC1 cDNA that includes one of its own introns to improve expression from the plasmid (Figure 9.

A fourth advantage of our approach is the ability to encode more than one gene on a plasmid to enable delivery of more than one protein product to a target tissue/cell (51, 52). This should ensure that a target tissue expresses all desired proteins with the expectation of a more efficient induction of immune response. A double cistron vector has been constructed, and we have shown that it is capable of expressing mouse or human IL-12. IL-12 is a protein comprised of two subunits that must be co-expressed in the same cell in order for the mature molecule to be produced. The two protein subunits are encoded by different genes, and we have shown in tissue culture that a double cistron

vector encoding both genes results in more effective production of the mature protein than using two plasmids which encode either gene alone (51, 52).

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We wished to explore the epitope spreading phenomenon further, specifically to learn if DNA vaccination followed by just a single tumor challenge with MUC1+ cells

would give rise to epitope spreading. Animals were vaccinated according to the groups shown in Figure 10. Vaccination with pMUC1/pIL-18 is the only regimen that results in significant protection (8/18 mice) compared to the empty vector group (p=0.007). Tumor weights are likewise significantly smaller in this group versus the other three groups (Figure 11). These results confirm the previous data demonstrating that the combination of pMUC1 and pIL-18 offer better protection against tumor challenge, and also cause a significant reduction in tumor weight in those animals that still develop tumors. Further, the data indicate that the combination of the two plasmids allows one to break tolerance to the MUC1 self antigen in the MUC1 transgenic mice.

The 8 protected mice from the pMUC1/pIL-18 group, and the 3 protected mice from the pMUC1-only group were challenged with MUC1$^+$ tumor cells (Figure 12). Only 1/15 control naïve animals survived tumor challenge, whereas 4/8 and 2/3 vaccinated animals remained tumor free. This result indicates that epitope spreading occurs with the immune response generated by the DNA vaccination and the first tumor challenge. Further, the fact that epitope spreading occurs in the pMUC1-only group suggests that IL-18 may not be required for this phenomenon to occur.

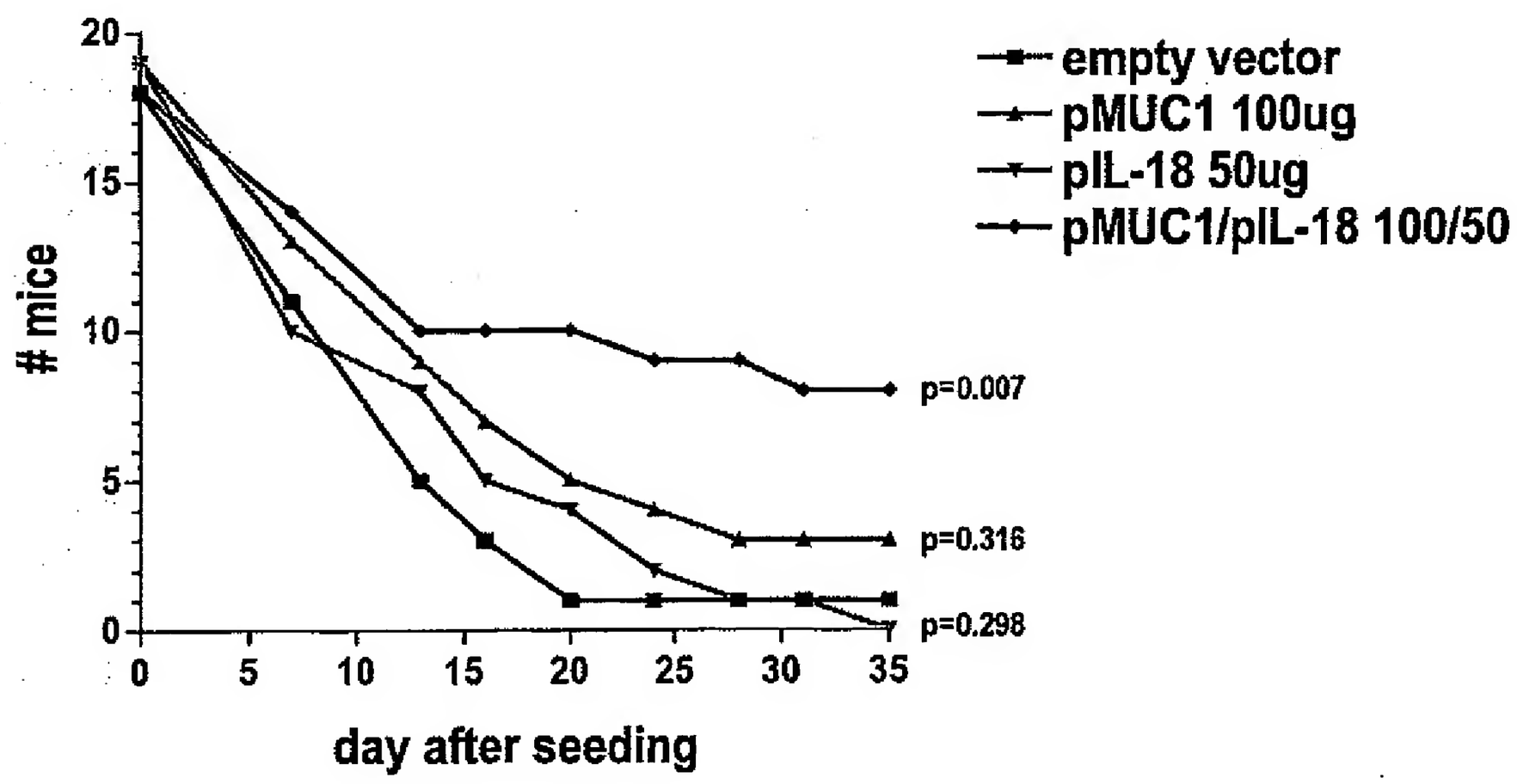

Figure 10. Tumor incidence in female MUC1 transgenic mice vaccinated with DNA as indicated in the legend, and subsequently challenged with MUC1$^+$ tumor cells. Only

the group vaccinated with pMUC1/pIL-18 shows significantly improved protection from tumor challenge (p=0.007).

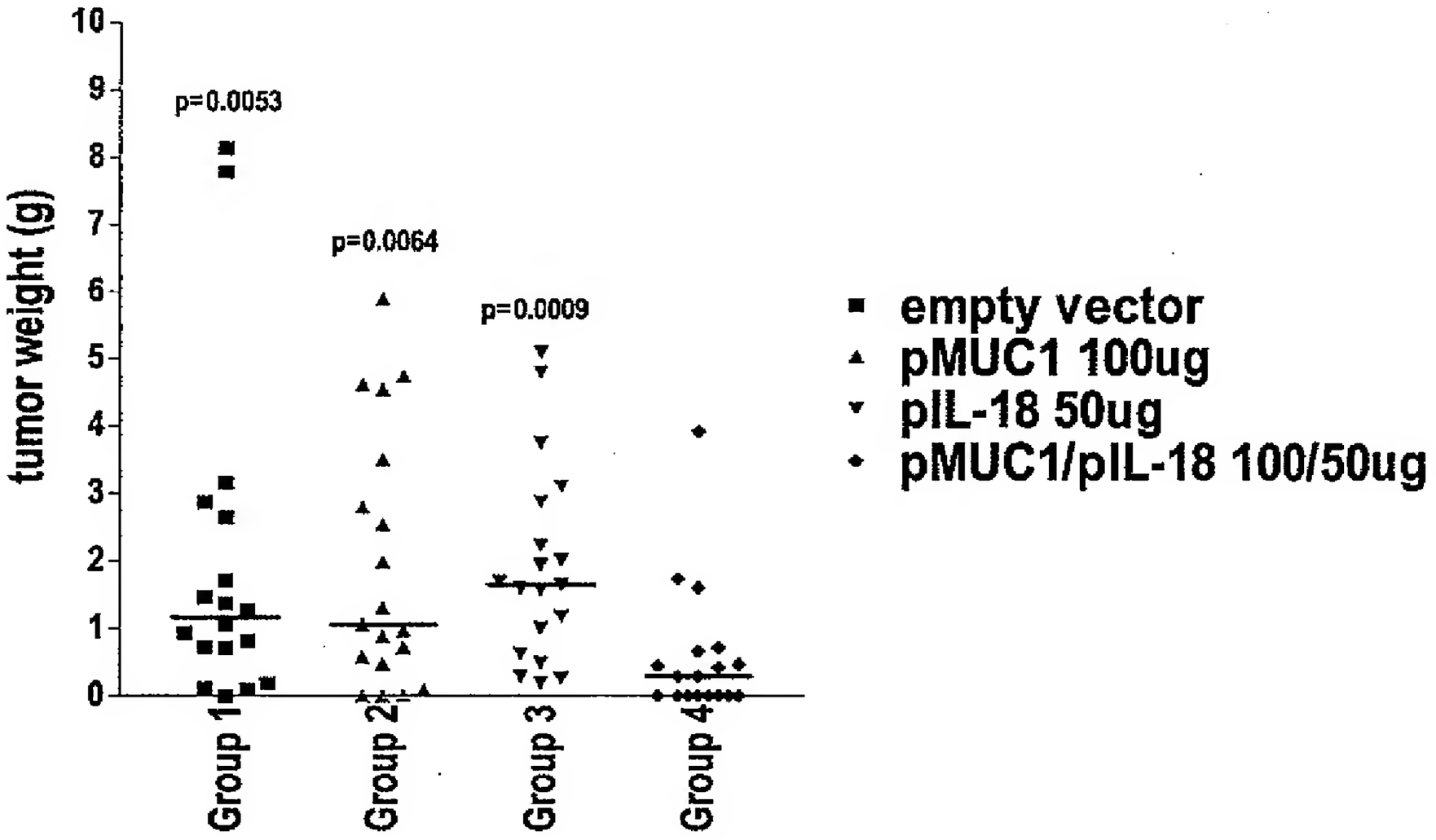

Figure 11. Media tumor weights at study end, from animals shown in Figure 1. Media tumor weight for group 4 is significantly different from those in the other groups.

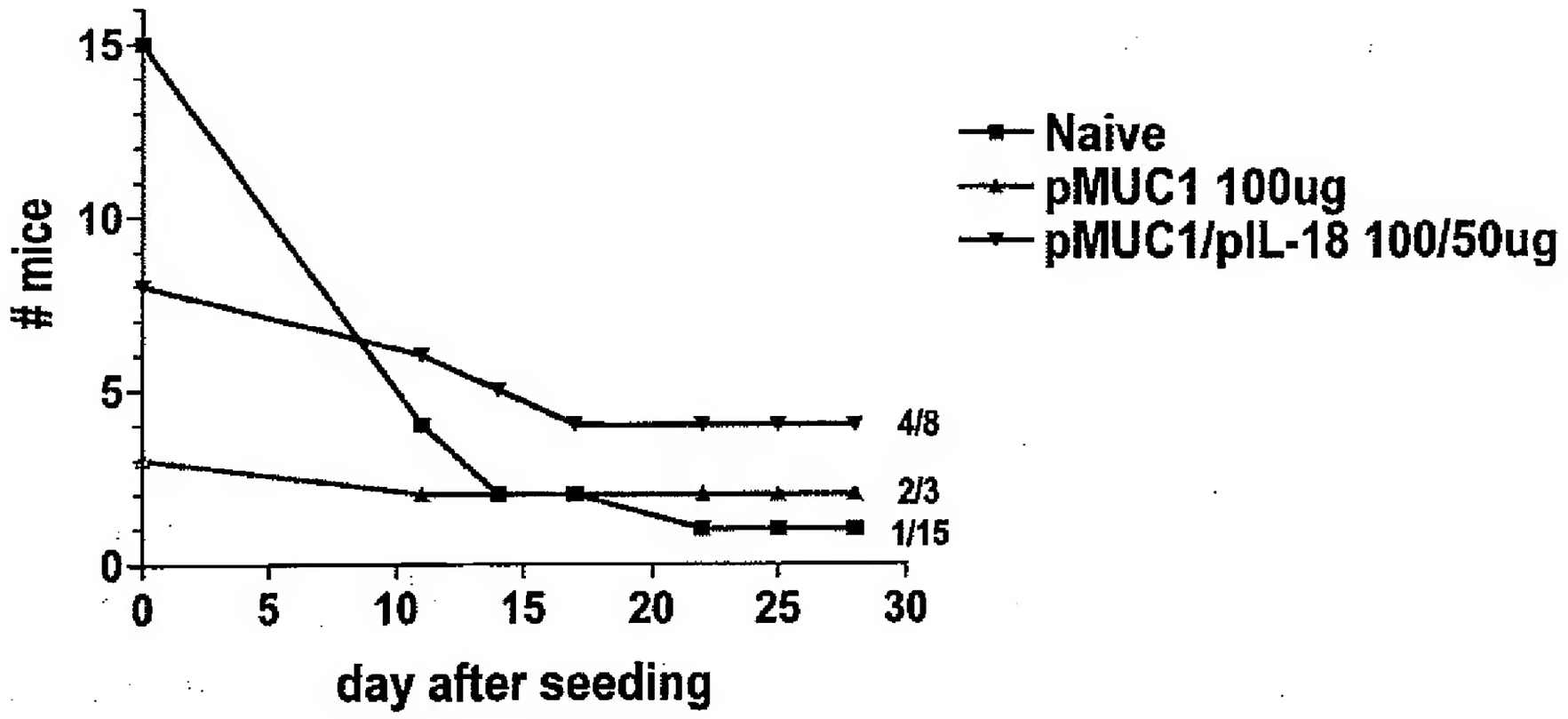

Figure 12. Rechallenge of protected mice from Figure 1 with MUC1$^-$ tumor cells.

Experimental conditions for above: Female MUC1 transgenic mice were vaccinated in Figure 12 with the indicated quantities of plasmids, on day 0, 14, and 21. Mice were challenged with $1.5x10^5$ MISA cells on day 28. They were monitored for tumor incidence, and tumor weights were measured at study end (Figure 11). The surviving mice from Figure 11 were challenged with $3x10^5$ MC38 cells 45-47 days after the initial tumor challenge (Figure 12).

Tumor protection studies in male MUC1 transgenic mice

We have tested whether vaccination of male MUC1 transgenic mice with pMUC1 plasmid can induce a protective immune response upon challenge with MISA cells. Male mice were vaccinated on day 0, 14 and 21 with various doses of DNA, then challenged on Day 28 with $1.5x10^5$ MISA tumor cells (Figure 13). In the control group, nearly all mice (9/10) succumbed to tumors. Male mice vaccinated with 150ug of pMUC1 showed good protection (6/10; p=0.019), and mice vaccinated with 100ug pMUC1 showed protection in 3/9 mice (not significant). Lower doses of pMUC1 did not result in any tumor protection. It appears that the pMUC1 plasmid alone can offer significant benefit in reducing tumor incidence, at high dose.

Tumor weights are shown in Figure 14. Again, the tumor weights in the highest dose group show a significant difference from the control group (p=0.015). This result suggests that the vaccination also helps to control growth of the tumor cells in the mice that still develop tumors.

To learn if the anti-tumor response was long-lived, the male mice that did not develop tumors (Figure 13) were rechallenged with $1.5x10^5$ MISA cells on day 39 after the first tumor challenge. As shown in Figure 15, 3/6 and 1/3 of the pMUC1 vaccinated mice remained protected after the rechallenge, suggesting that some animals did develop a long-lived recall response to the tumors.

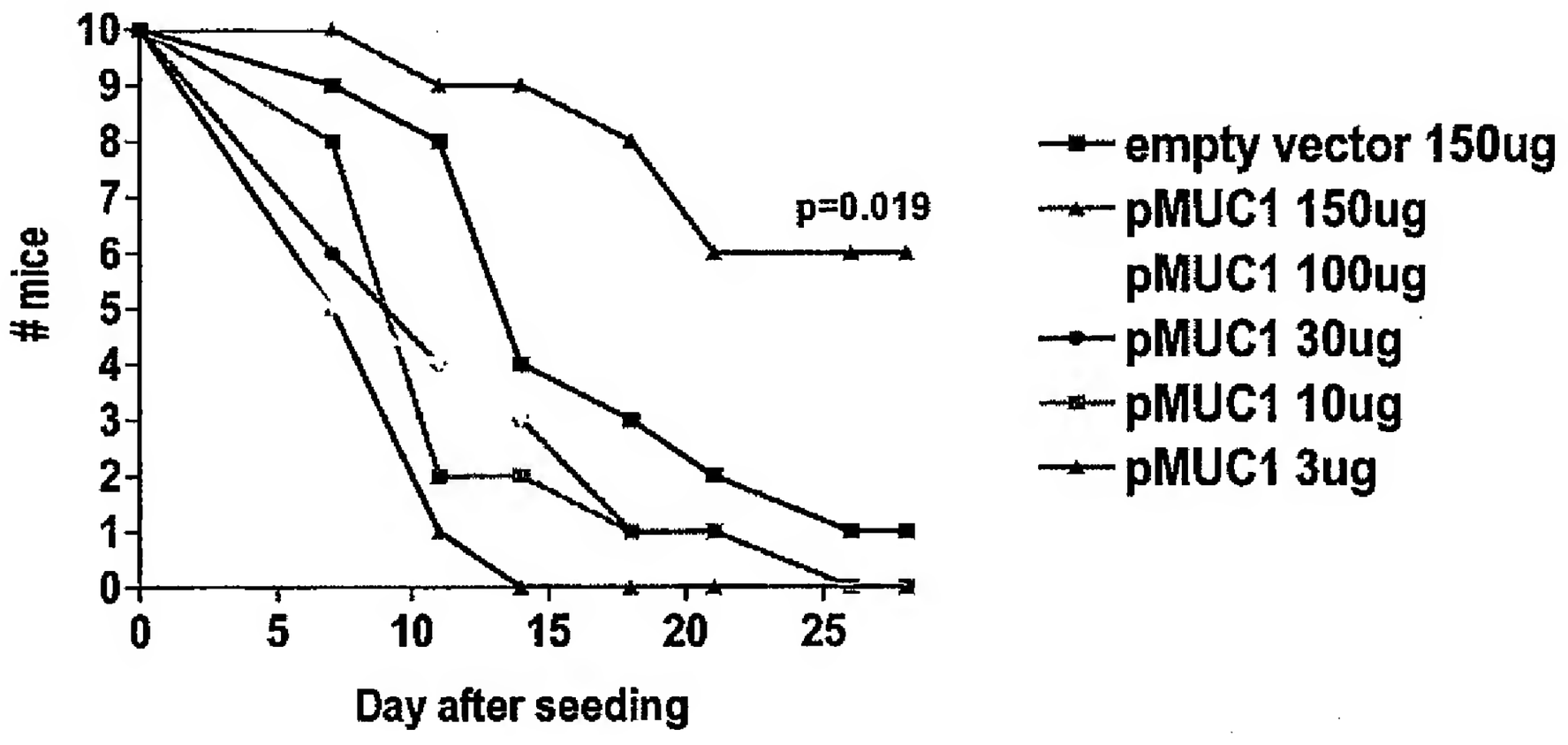

Figure 13. Tumor incidence in male mice vaccinated with pMUC1 or empty vector, followed by tumor challenge.

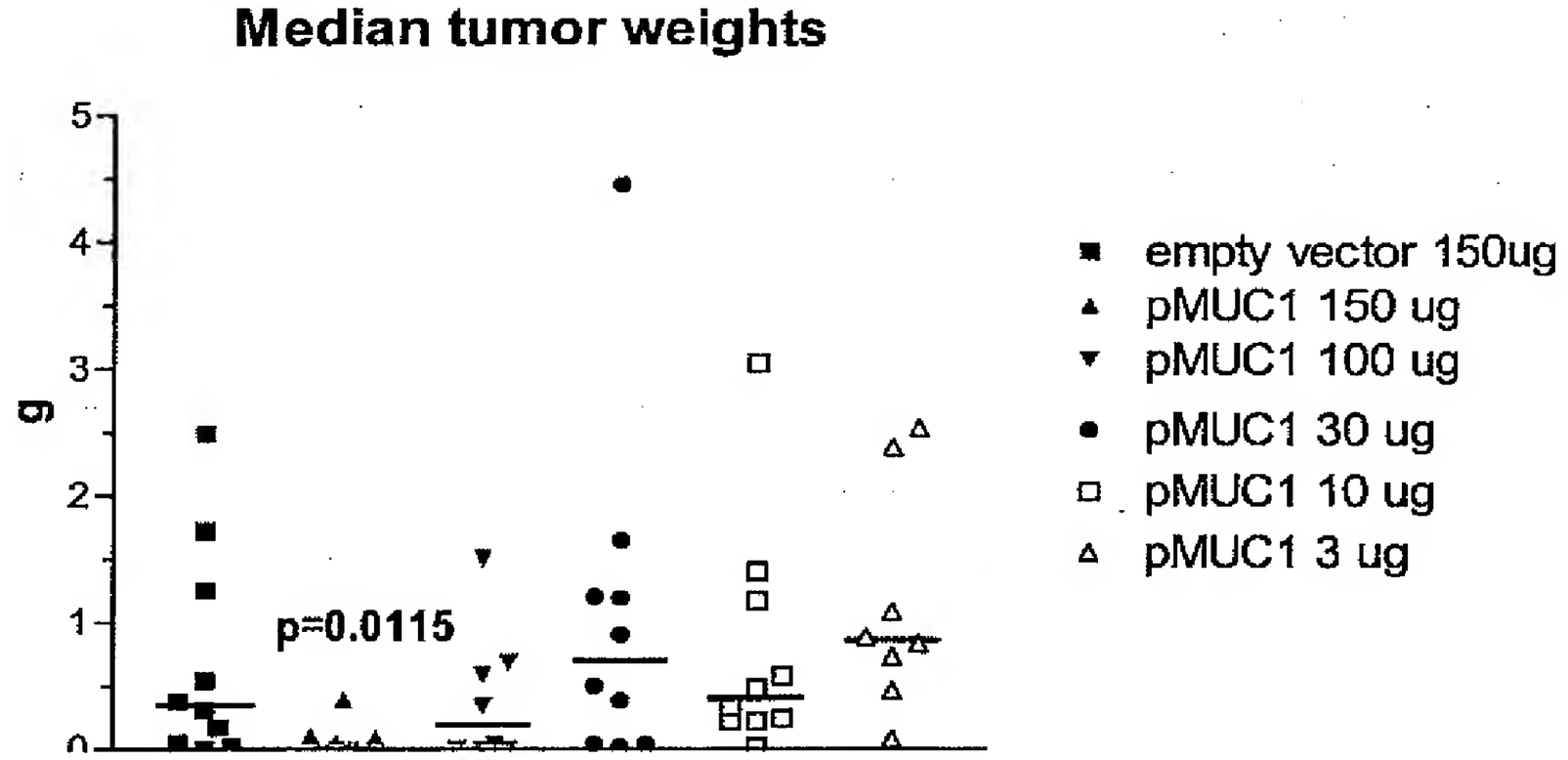

Figure 14. Tumor weights in male mice vaccinated with pMUC1.

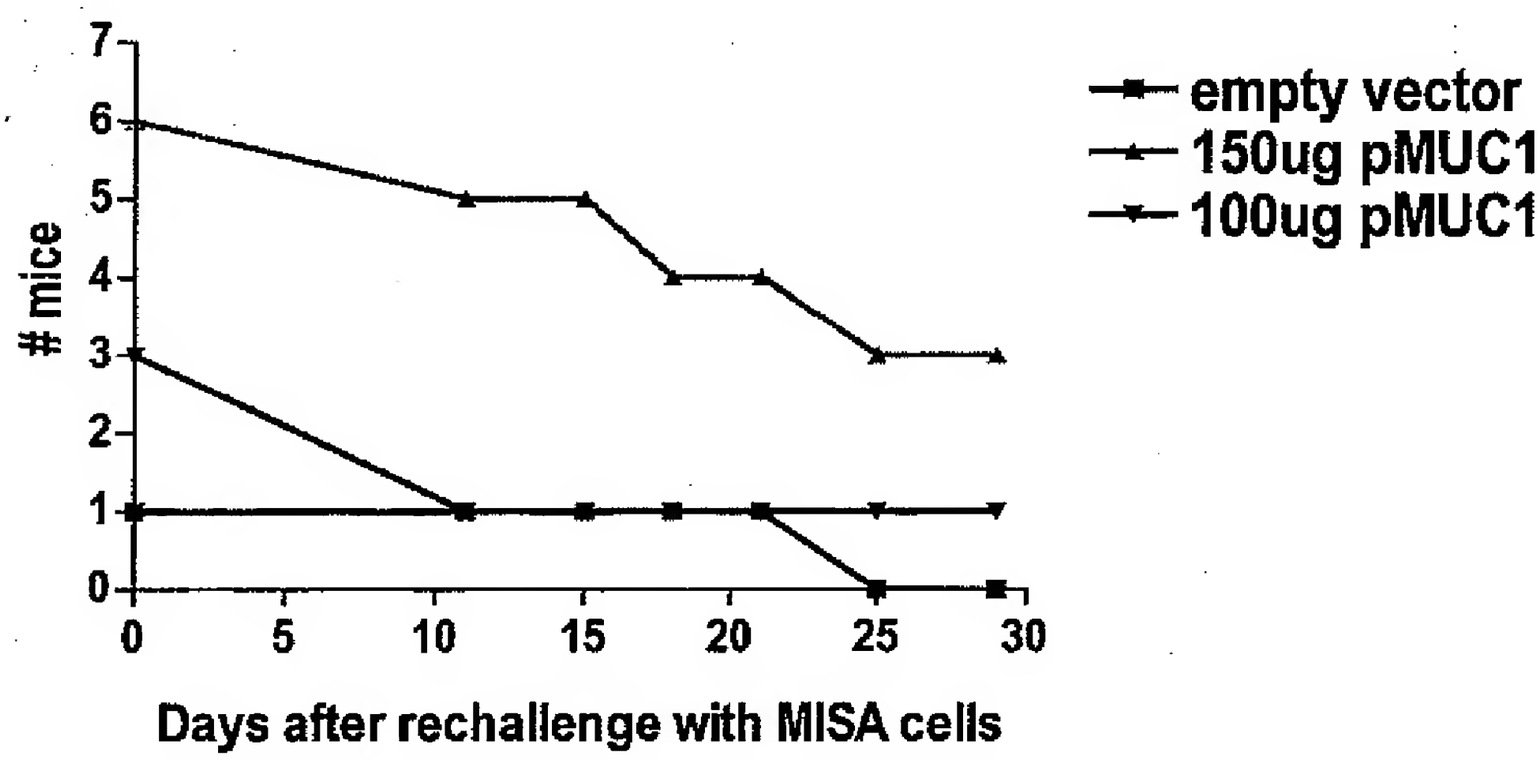

Figure 15. Tumor incidence in male mice rechallenged on the opposite flank with MUC1+ tumor cells.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

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WHAT IS CLAIMED IS:

1. A nucleic acid vaccine, comprising

(a) at least one polynucleotide encoding at least one antigenic portion of at least one amino acid sequence comprising or encoded by at least one of SEQ ID NOS:1-47 or variants thereof, or a nucleic sequence complementary thereto; and

(b) at least one polynucleotide encoding at least one adjuvant encoding portion of at least one amino acid sequence comprising or encoded by at least one of SEQ ID NOS:60-77 or variants thereof, or a sequence complementary therero.

2. A nucleic acid vaccine according to claim 1, wherein said antigen is selected from at least one of MUC-1, PSA, or KLK2.

3. A nucleic acid vaccine according to claim 2, wherein said MUC-1 amino acid sequence is selected from at least one antigenic portion of at least one of SEQ ID NOS:20, 22, 26, 28, 30, 32, 34, 35, 37, 39, 41, 43, and 47.

4. A nucleic acid vaccine according to claim 2, wherein said PSA amino acid sequence is selected from at least one antigenic portion of at least one of SEQ ID NOS:1, 4-10, 12 and 14-15.

5. A nucleic acid vaccine according to claim 2, wherein said IL-18 amino acid sequence is selected from at least one antigenic portion of at least one of SEQ ID NOS:64, 65, 69, 70-71, 74-75 and 77.

6. A nucleic acid vaccine according to claim 1, wherein the vaccine further comprises at least one promoter sequence controlling the expression of said antigen encoding polynucleotide.

7. A nucleic acid vaccine according to claim 2, wherein the promoter is at least one cytomegalovirus immediate early (CMV) promoter.

8. A nucleic acid vaccine according to claim 2, wherein the promoter is at least one dihydrofoliate reductase (dhfr) promoter.

9. A nucleic acid vaccine according to claim 2, where the promoter is at least one early or late SV40 promoter.

10. A nucleic acid vaccine according to claim 1, comprised of a nucleic acid vector.

11. A nucleic acid vaccine according to claim 1, comprised of a host cell

12. A nucleic acid vaccine according to claim 1, comprised of viral vector.

13. A composition comprising a nucleic acid vaccine according to claim 1.

14. A tumor/adjuvant vaccine composition comprising a nucleic acid vaccine according to claim 1 and a pharmaceutically acceptable carrier or diluent.

15. A nucleic acid vaccine composition of claim 11, further comprising an additional adjuvant and/or cytokine encoding sequence or component of the composition which enhances a nucleic acid vaccine immune response to at least one cancer associated tumor protein in a mammal administered the vaccine composition.

16. A method for eliciting an immune response to a cancer associated tumor protein in a mammal that is prophylactic for a cancer associated tumor protein, comprising administering to a mammal a nucleic acid vaccine according to claim 1.

17. A method for eliciting an immune response to a cancer associated tumor protein in a mammal for therapy of a tumor-associated pathology, comprising administering to a mammal a nucleic acid vaccine according to claim 1.

18. A method according to claim 13, further comprising priming or boosting a humoral or cellular immune response, or both, by administering an effective amount of at least one of said nucleic acid vaccine.

19. A method according to claim 14, further comprising priming or boosting a humoral or cellular immune response, or both, by administering an effective amount of at least one of said nucleic acid vaccine.

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WO 2003/031569 A3

(54) Title: NUCLEIC ACID VACCINES USING TUMOR ANTIGEN ENCODING NUCLEIC ACIDS WITH CYTOKINE ADJUVANT ENCODING NUCLEIC ACID

(57) Abstract: Nucleic acid vaccines are provided that comprise at least one tumor antigen encoding nucleic acid and at least one cytokine adjuvant encoding nucleic acid for prophylaxis or treatment of tumors. The viral vaccines of the invention are optionally combined or additionally administered with a recombinant virus or DNA vaccine booster.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/29640

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/70, 48/00; C12N 15/63; C07H 21/04
US CL : 514/44; 424/93.21; 435/320.1, 455; 536/23.1, 23.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/44; 424/93.21; 435/320.1, 455; 536/23.1, 23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/61068 A1 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 02 December 1999, see the entire document, particularly pages 3-5; page 12, lines 24-31; page 13, lines 17-25; and SEQ ID NO:1.	1-2, 4, 6-10, 12-14 and 16-19
X,P	WO 02/40059 A2 (AMERICAN FOUNDATION FOR BIOLOGICAL RESEARCH, INC.) 23 May 2002, see the entire document, particularly pages 2-6; pages 64-68; and SEQ ID NO:1.	1-2, 4 and 6-19
X	KIM et al. Engineering enhancement of immune response to DNA-based vaccines in a prostate cancer model in Rhesus Macaques through the use of cytokine gene adjuvants. Clin. Cancer Res. March 2001, Vol. 7, pages 882s-889s, see the abstract.	1-2, 4, 6-7, 10, 13-14, 16-19
A	EP 0 845 530 A2 (KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO) 03 June 1998, see the entire document.	1-2, 4 and 5-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
01 October 2004 (01.10.2004)

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INTERNATIONAL SEARCH REPORT

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-2 and 4-19 with SEQ ID NO:1 and SEQ ID NO:60

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

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INTERNATIONAL SEARCH REPORT

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-2 and 4-19, drawn to a nucleic acid vaccine of claim 1, wherein the selected antigen is PSA and methods for eliciting an immune response to a cancer associated tumor protein in a mammal using the same.

Group II, claims 1-3 and 5-19, drawn to a nucleic acid vaccine of claim 1, wherein the selected antigen is MUC-1 and methods for eliciting an immune response to a cancer associated tumor protein in a mammal using the same.

Group III, claims 1-2 and 5-19, drawn to a nucleic acid vaccine of claim 1, wherein the selected antigen is KLK2 and methods for eliciting an immune response to a cancer associated tumor protein in a mammal using the same.

Additionally, each of the aforementioned Groups I to III contains inventions which are not linked as to form a single general inventive concept under PCT Rule 13.1, depending on which SEQ ID NOs: 1-47 is used in combination with which SEQ ID NOs: 60-77. Applicants are requested to elect one of SEQ ID Nos: 1-47 and one of SEQ ID Nos:60-77. The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking Groups I to III appear to be that they all relate to a nucleic acid vaccine comprising: (a) at least one polynucleotide encoding at least one antigenic portion of at least one amino acid sequence comprising or encoded by at least one of SEQ ID NOS:1-47 or variants thereof (sequences encoding PSA, KLK2 and MUC-1), and (b) at least one polynucleotide encoding at least one adjuvant encoding portion of at least one amino acid sequence comprising or encoded by at least one of SEQ ID NOS:60-77 or variants thereof (IL-18 and variants).

However, Kim et al. (Clin. Cancer Res. 7:882s-889s, March 2001) already teach a vaccine composition comprising an expression cassette encoding PSA under the control of cytomegalovirus promoter and an IL-18 gene for enhancing immune responses against prostate cancer in mouse and rhesus macaque animal models (see abstract, particularly page 885s, col. 2, lines 4-6; page 886s, col. 2, second paragraph).

Therefore, the technical feature linking the inventions of Groups I to III does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not differentiate the claimed subject matter as a whole over the prior art. Since according to Rule 13.2 PCT the presence of such a common or corresponding special technical feature is an absolute prerequisite for unity of invention to be established, and given that there does not appear to be any other technical feature common in the claimed subject matter as a whole which might be able to fulfill this role, the currently claimed subject matter lacks unity of invention according to Rule 13.1 PCT.

Consequently, the claimed subject matter was broken up into the aforementioned Groups of Inventions.

The nucleic acid vaccine of Groups I to III are different chemically and structurally one from the others, and that they require different technical consideration for attaining the desired therapeutic effects in the method of uses. For example, the nucleic acid vaccine of Group I does not require the presence of any polynucleotide encoding MUC-1 or KLK2 of the present invention. Similarly, the nucleic acid vaccine of Group II does not require the presence of any polynucleotide encoding PSA or KLK2 of the present invention. The nucleic acid vaccine of Group III does not require the presence of any polynucleotide encoding PSA or MUC-1 of the present invention. Moreover, PSA, MUC-1 and KLK2 are not structurally related, nor do they have the same biochemical activities.

Additionally, SEQ ID NOs: 1-47 lack the same or corresponding special technical feature because each SEQ ID NO. is different one from the others depending upon the presence or absence of signal sequences, introns, encoded antigens (e.g., PSA, KLK2 or MUC1), sources (human, rhesus macaque), CTL epitopes, and thus each SEQ ID NO. is different structurally and has different biochemical properties one from the others. Similarly, SEQ ID NOs: 60-77 lack the same or corresponding special technical feature because each SEQ ID NO. is different one from the others depending upon whether the IL-18 sequences contain signal sequences, introns, variants having different IL-18 activity and derived from different sources (e.g., human rhesus macaque).

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Continuation of B. FIELDS SEARCHED Item 3:
APS, DIALOG, MEDLINE, CANCER LIT., BIOSIS, EMBASE
Search terms: DNA vaccine, PSA, IL-18, prostate specific antigen, cytokine adjuvants.